Microbiological status of root canal after unsuccessful endodontic treatment

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SUMMARY

Introduction The main objective of endodontic treatment is to eliminate infection from root canal and prevent re-infection by three-dimensional hermetic obturation of the canal system. Endodontic failure can occur due to inability of complete control and elimination of infection from the root canal.

The aim of this study is to investigate, by PCR technique, microbiological status of previously endodontically unsuccessfully treated teeth immediately after the removal of obturation material.

Material and Methods The analysis included 30 teeth indicated for endodontic retreatment. After removing previous root canal filling material, the bacteriological sample was taken by sterile instrument (# 15) and paper points. Standard PCR technique was used to analyze the incidence of E. faecalis, P. micros, P. intermedia, P. endodontalis and A. actinomycetemcomitans.

Results Positive bacteriological findings were registered in 80% of cases, while bacteria were not identified in 20% of all samples (all taken from the root canals without significant changes in periapical tissue). From 24 canals with identified bacteria, 17 had affected apical periodontium. The most dominant microbe in root canals with positive bacteriological finding was E. faecalis (83.3% of the canals) and P. intermedia (75%). In case of teeth with chronic periapical changes, the most common was E. faecalis (94%) and P. intermedia (82.3%).

Conclusion The presence of periapical lesions significantly affects microbiological status of endodontically treated teeth. The presence of bacteria was confirmed in most teeth with periapical lesions, while the most frequently identified bacteria were E. faecalis, P. intermedia and P. micros.

Keywords: PCR; E. faecalis; endodontic failure

INTRODUCTION

The main objective of endodontic treatment is to eliminate infection from the root canal and prevent re-infection by three-dimensional hermetic obturation of the canal system. However, sometimes even properly conducted endodontic treatment can fail. It has been confirmed that the outcome of endodontic treatment largely depends upon the quality of endodontic procedures and possibility of eliminating infection from root canal system before obturation [1, 2]. Due to the complexity of canal systems their cleaning is difficult, therefore bacteria may remain in inaccessible parts of the canal, especially in the apical portion.

Persistant infection in the apical third is most often a result of inadequate completion of endodontic treatment i.e. non-aseptic conditions with insufficiently extended and poorly designed access cavities, insufficient dimension of instrumentation, inadequate hermetic obturation or microleakage due to inadequate temporary or definitive restorations [3]. However, number of infections may persist as asymptomatic periapical radiolucency even if endodontic procedure is properly implemented. The reason is usually complex anatomy of the root canal system with regions that cannot be adequately treated and obturated by existing instruments, materials and techniques [4]. Some studies have shown that certain parts of the root canal space remain untouched during chemo-mechanical instrumentation regardless of the preparation technique or instruments used [5]. Untreated parts of the root canal can contain bacteria and necrotic tissues even when obturation seems to be radiographically correct [4]. It is believed that endodontic failure is caused by inability of complete control and elimination of infection in the root canal.

The aim of this study was, using PCR technique, to investigate microbiological status of endodontically treated teeth with persistent infection immediately after the removal of obturation material.

METHODS

Material for microbiological tests was obtained by taking samples from 30 patients who had root canal treatment done earlier but they needed retreatment. After obtaining dental history, and taking periapical radiographs, patients...
were clinically examined and failure of the initial endodontic treatment was diagnosed.

Microbiological study included 30 teeth (8 multi-rooted and 22 single-rooted) indicated for endodontic retreatment. Primary endodontic therapy was performed 12 months ago in 2 cases, 1-5 years in 8 cases, while for 20 teeth, primary endodontic treatment was done >5 years ago. All 30 teeth had inadequate obturation and that was the failure criterion for which patients needed retreatment. Poor quality of obturation was assessed as short filling (in 22 teeth), as "forgotten" canal (in 7 teeth), or separated instrument (in 1 tooth). Adequate restoration or prosthetic restorations (crowns) were observed in 13 teeth, 7 teeth were without coronal restoration for a longer period of time and 10 teeth did not have proper restoration. The presence of symptoms such as pain, swelling, presence of a fistula, sensitivity to percussion or pain when biting were observed in 10 patients, while the remaining 20 had no clinical signs or symptoms.

Status of apical periodontal tissue was evaluated by PAI index, where completely healthy periodontium (PAI 1) was radiographically registered in 6 cases; PAI 2 score (small changes in the bone structure that are not pathognomonic for apical periodontitis) was recorded in 7 cases; PAI 3 score (which includes changes in bone structure with decalcification characteristic for apical periodontitis) was registered in 13 teeth; PAI 4 (which represents periodontitis with clearly defined zone of radiolucency) was noted in 3 cases; the highest score of PAI 5 (advanced periodontitis with signs of exacerbation and expansion of the bone), was registered only in 1 case. Microbiological process involved first the removal of hard and soft deposits from teeth, restorations and decay and placing a rubber dam. Disinfection of the operative field was done with 30% hydrogen peroxide solution and 2.5% sodium hypochlorite solution, which is then inactivated with 5% hydrogen peroxide solution and 2.5% sodium hypochlorite solution. With the aim to isolate DNA, 100 ml of redistilled water was added in each micro-tube with paper points. Isolation of total bacterial DNA (Gram positive and Gram negative bacteria) was performed using commercial kit QIAamp DNA Mini Kit (Qiagen). After application of isolation protocol, bacterial DNA was dissolved in 100 ml of elution buffer.

The incidence of the following bacteria was analyzed by classical PCR: Enterococcus faecalis, Peptostreptococcus micros, Porphyromonas endodontalis and Actinobacillus actinomycetemcomitans. The sequences of used primers; the temperature profile and length of PCR products are shown in Table 1. In each PCR reaction, simultaneously with the test samples, positive and negative controls were used to avoid false positive and negative results. Reaction mixture of PCR with 25 ml volume was made up of the following components: 13 ml dH2O shall, 2.5 ml PCR buffer, 1.5 ml of 25 mM MgCl₂, 1 ml of dNTP, per 1 ml of F and R primers, 0.2 ml of Taq polymerase and 5 ml of bacterial DNA.

The effectiveness of PCR reaction was measured by electrophoresis on a vertical 8% polyacrylamide gel (PAA) in 1XTBE buffer, at constant voltage of 200 V for a period of 30 min. Visualization of PCR products was performed by staining with ethidium bromide.

RESULTS

PCR technique was used to analyze the presence of the following microorganisms: E. faecalis, P. micros, P. inter-

| Microorganism | Primer | Temperature Profile | PCR Product |
|---------------|--------|---------------------|-------------|
| Enterococcus | F, TACTGACAACACCCATTCATGATG R, AACTCTGCAACCACTGGAAC | 95°C 3min, 95°C 45s, 55°C 1min, 72°C 1min, X5 cycles 72°C 5min | 112bp |
| Faecalis | F, TCAGTTGATCCGTCGAG R, TATATCTGGATCTTGTC | | 204bp |
| Peptostreptococcus | F, AGAATTTGTGATGTCGTCAG R, TATATCTGGATCTTGTC | | 259bp |
| Micros | F, GCATTGACCAAGAGATCTGGTCGTCAG R, GCGTTTACTTCACCACAAA | | 627bp |
| Prevotella | F, CAGACAGTCACTGAGTACTC R, CACCACTGTCACCTGTC | | 500bp |
| Intermedia | F, CAGAGTACCAACTGAGTACTC R, CACCACTGTCACCTGTC | | 627bp |
| Gliden drills | F, GCTAATGGCCGTAAGCTGCGG R, ATTTACACGCTCATTAAAGGT | | 500bp |
Positive bacteriological findings were registered in 80% of cases, while the bacteria were not identified in 20% of samples. All negative samples were taken from the root canal without significant changes in the apical periodontal tissue (PAI 1, 2) while 17 out of 24 canals with identified bacteria belonged to the teeth with damaged apical periodontium (PAI 3, 4, 5) (Table 2). All samples taken from the root canals with chronic periapical lesions were positive for bacteria (100%).

Isolated bacteria mainly belonged to E. faecalis (66.6%) followed by P. micros (46.6%), P. endodontalis (26.6%) and A. actinomycetemcomitans (10%) (Table 3). The most dominant microorganism in root canals with positive bacteriological findings was E. faecalis (83.3%), followed by P. intermedia (75%) and P. micros (58.3%) (Table 4). In the group of teeth with healthy apical pericapical tissue, all bacterial species (except A. actinomycetemcomitans which was not detected in any of the samples) were equally represented or were identified in 57.7% of canals. In the case of teeth with chronic periapical changes, the most common isolation was E. faecalis identified in 94% of the canals, then P. intermedia that was present in 82.3% of samples (Table 4).

In regards to the number of bacterial species contained in a single sample, monoinfection was registered in 13.3% of cases (E. faecalis was present in half of the canals), while the most common bacteria identified in 33.3% of canals were 3 bacterial species per one canal E. faecalis, P. intermedia and P. micros (Table 5). It was observed that the samples taken from the tooth with healthy periodontal tissues around the root tip, showed mainly absence of bacteria or the presence of 1 or 2 bacterial species, while in the case of chronic periapical changes, in more than a half of samples, the presence of 3 or more bacterial species was identified (Table 6).
Correlation between the presence of certain symptoms after the initial endodontic treatment and findings of specific bacterial species in root canal are shown in Table 7. *E. faecalis* and *P. intermedia* were detected in all patients with pain, teeth sensitive to percussion and pain when biting, as well as the half of the samples taken from the root canal with sinus tract and swelling. *P. micros* was identified in 66.6% of patients with pain, 50% of patients with swelling, sinus tract and percussion sensitivity and 33.3% of patients with pain when biting. The presence of *P. endodontalis* was confirmed in 33.3% of the canals registered with spontaneous pain and 25% with sensitivity to percussion. *A. actinomycetemcomitans* was identified in 50% of the canals with sinus tract, 33.3% of patients with pain when biting and 12.5% of the samples taken from the root canal of patients with sensitivity to percussion (Table 7).

**DISCUSSION**

The analysis included 30 teeth that needed retreatment regardless of its causes - prosthetic indications, incidental finding or the patient reported having problems. In order to prevent contamination of canal samples, strictly aseptic conditions were conducted by the current protocol for disinfection of working fields [6-9]. This involved decontamination and disinfection of the operative field with 30% hydrogen peroxide solution and 2.5% sodium hypochlorite followed by inactivation of 5% sodium thiosulfate so that its remains would not affect the sample taken [7]. Also, complete removing of the root canals filings was done purely mechanically without use of any solvents, lubricants or irrigants. The micro-tubes with microbiological material were stored at -20°C, for no longer than a month.

PCR (Polymerase Chain Reaction) is a modern, fast (it takes a few hours), and a simple method for identification of microorganisms. It is extremely sensitive and highly specific. Theoretically, it is possible to demonstrate the presence of only single bacterial cell (living or dead) in the sample, although the number of 10 cells is considered to be the lowest limit for detection (e.g. 100 viable cells are necessary for one method of bacteria cultivation) [9]. Identification is based on in vitro amplification of target DNA fragment, which can be repeated up to a billion times. Because its detection works on the basis of genotypic structure, rather than phenotypic characteristics of microorganisms, identification is very reliable and precise. It is possible to identify those bacterial species that cannot be cultivated in vitro or cases where the number of cells in examined material is very small [9]. However, the PCR method of identification of microorganisms has its limitations. Firstly, the species that are not targeted or detected by other methods cannot be identified. Precisely determined conditions are required for reaction with specific primer pair that can detect only particular and specific bacterial species. It should be noted that PCR method can not determine if identified cell is alive or dead. Another limitation of the conventional PCR method is its inability to quantify the number of bacteria. This can be overcome by “real-time” PCR method where the number of bacterial cells can be determined [10].

Positive bacteriological findings were registered in 80% of cases, while no bacteria were identified in 20% of samples. All samples taken from root canals with chronic periapical lesions were positive for presence of bacteria (100%). Also, all negative samples were taken from root canal with healthy periapical tissue. This confirmed the hypothesis that without bacteria, there is no infection in periapical tissues, and consequently there is no failure of endodontic treatment. Similar results were obtained by Siqueira et al. [5], Gomes et al. [11,12], Roca et al. [13], Sedgley et al. [14] and Sakamoto et al. [15].

However, 23% of positive bacteriological samples were taken from root canals where periapical tissue had PAI score 2 (periapical radiolucency) with no pathognomonic signs of chronic inflammation. Kaufman et al. [16] and Olette et al. [17] reported the presence of bacteria in the root canal without any periapical changes detected radiographically. The explanation may lie in the fact that two-dimensional radiography is not sufficiently accurate to diagnose apical periodontitis with less destroyed bone tissue [18]. Also, it could be that small number and low virulent bacteria are present. Only if present in higher numbers and pathogenic bacteria persisting after primary endodontic treatment can cause or maintain periapical inflammation [2]. However, there is a dilemma whether bacteria remained after primary endodontic treatment (persistent infection) or they are the result of re-infection (secondary infection). In the recent years, research has pointed out the importance of proper coronal restora-

| Symptom          | Pain Bol     | Swelling Otok | Sinus tract Fistula | Percussion sensitivity Perkutorna osetljivost | Pain when biting Bol na zagrižaj |
|------------------|--------------|---------------|---------------------|---------------------------------------------|---------------------------------|
| Microorganism    | N            | %             | N                   | %                                          | N                               | %                               |
| E. faecalis      | 3            | 100           | 1                   | 50                                         | 1                               | 100                             |
| P. micros        | 2            | 66.6          | 1                   | 50                                         | 1                               | 50                              |
| P. endodontalis  | 1            | 33.3          |                     |                                             | 2                               | 25                              |
| P. intermedia    | 3            | 100           | 1                   | 50                                         | 1                               | 100                             |
| A. actinomycetes | 1            |               |                     |                                             | 1                               | 12.5                            |
| Number of patients with Symptoms Broj pacijenata sa simptomima | 3 | 2 | 2 | 8 | 3 |

Table 7. Distribution of bacteria in correlation with the presence of certain symptoms Tabela 7. Distribucija bakterija u odnosu na prisustvo određenih simptoma
tion in preventing re-infection of endodontic space with opinion that secondary infection is important cause of endodontic treatment failure [1, 19]. The most common microorganism in the canal system with positive bacteriological findings was *E. faecalis* followed by *P. intermedia* and *P. micros* accounting for 58.3%. *E. faecalis* was identified in 94% of root canals with chronic periapical lesions (PAI 3, 4, 5) which is similar to findings of Sedgley et al. who also used PCR identification method. They used 48 samples and showed that the incidence of *E. faecalis* finding was 90% [14]. Gomes et al. used PCR to analyze microbiological status of previously filled canals with periapical lesions and came to the conclusion that *E. faecalis* was present in 90% of bacteriologically positive root canals followed by *P. micros* 59%, *P. gingivalis* 41%, *P. endodontalis* 26% and *P. intermedia* 13% [12]. This high percentage of *E. faecalis* was probably the result of its numerous and diverse virulence factors and extraordinary ability to survive. This microorganism is small; it easily penetrates into dentinal tubules and has a good adherence to collagen [20]. It is resistant to calcium hydroxide [21] and has the ability to survive (as a single species) in dentinal tubules without the support of other bacteria [22]. Also, it has the ability to survive without nutrients and recover easily when they become available in the form of serum (the origin of alveolar bone and periodontal ligament) [23].

The results of this study confirmed the presence of *E. faecalis* in 57.7% of root canals without changes in apical periodontal tissue. However, *Pintermedia, P. endodontalis* and *P. micros* were also identified in 57.7% of root canals which leads to the conclusion that *E. faecalis* is not the only microorganism responsible for failure of endodontic treatment. Williams et al. showed that *E. faecalis* can survive all stages of endodontic treatment because RT-PCR detected its presence in samples taken immediately after instrumentation and irrigation as well as after medication [24].

Our research indicated that remaining microorganisms could be present in root canals due to inadequate coronal seal. In 70% of teeth with healthy periodontal apical tissue, coronal restoration did not show satisfactory quality that opened door for secondary infection of endodontic space. In addition, although present in root canal, the bacteria had no effect on periapical tissue. They probably remained blocked and trapped in dentinal tubules or root canal filling material blocked further progress to periapical tissues (microleakage).

Patients who took part in our study came from general dental practice and health centers where it was quite difficult to adhere to contemporary standards and good endodontic practice, therefore the incidence of residual bacteria after primary endodontic treatment was high. *Prevotella spp* and *Porphyromonas spp* (previously classified as *Bacteroides spp*) belong to the group of “black-pigmented bacteria” because in contact with agar, they form shiny, smooth colonies of gray or black. According to the new taxonomy, saharolytic Bacteroides spp species are classified as genus Prevotella, while asaharolytic species belong to genus *Porphyromonas*. Types of *P. intermedia, P.melaninogenica, P.denticola* and *P.dentalis* belong to gram-negative obligate anaerobes. Although they have limited ability of fermentation of amino acids and require the presence of hemin and menadione for growth, they can be observed in different parts of body (oral cavity, upper respiratory and urogenital system) [25]. Ruan et al. found that *P. intermedia* originating from oral cavity represents potential opportunistic microorganism associated with periodontal disease but also apical periodontitis due to its adhesiveness and competitiveness with surrounding microorganisms [25]. *P. endodontalis* is gram-negative microorganism associated with periodontitis, endodontic infections and gingivitis, and more frequently with symptomatic than asymptomatic infections in oral cavity [26].

One-third of patients who took part in the current study showed some clinical symptoms, but the most common symptom was sensitivity to percussion. The most common microorganisms present in the samples taken from such teeth were *E. faecalis* and *Pintermedia* (100%), followed by *Pmicros* (50%). Gomes et al. also found statistically significant relationship between *Pmicros* and tooth sensitivity to percussion [12] while Pinheiro et al. noticed association between *Pintermedia* and the presence of these symptoms [27]. *E. faecalis* and *Pintermedia* were detected in all patients with pain, tenderness to percussion and pain when biting, as well as one half of the samples taken from root canals of patients with swelling and fistula. *P. micros* was identified in 66.6% of patients with pain that is in accordance with the study of Pinheiro et al. who reported involvement of bacterial species *Pintermedia* and *Pmicros* in teeth where pain was present [27].

**CONCLUSION**

The presence of periapical lesions significantly affects microbiological status of endodontically treated teeth. The presence of bacteria in root canals was confirmed in most cases of unsuccessful endodontic treatments, while the most frequently identified bacteria were *E. faecalis*, followed by *Pintermedia, Pmicros* and *P. endodontalis*.

**REFERENCES**

1. Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J. 1995; 28:12–8. [PMID: 7642323]
2. Siqueira JF. A. Ethology of root canal treatment failure: why well-treated teeth can fail. Int Endod J. 2001; 34:1–10. [PMID: 11307374]
3. Nair PNR. On the causes of persistent apical periodontitis: a review. Int Endod J. 2006; 39:249–81. [DOI: 10.1111/j.1365-2591.2006.01099.x]
4. Nair PNR, Henry S, V Cano, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Dent. 2005; 99:231–52. [DOI: 10.1016/j.tripleo.2004.10.005] [PMID: 15660998]
5. Siqueira JF, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Dent 2004; 97:85–94. [DOI: 10.1016/s010921040403536] [PMID: 14716262]
6. Friedman S, Mor Ch. The success of endodontic therapy- and healing functionality. CDA J 2004; 32:493–503. [PMID: 15344440]

7. Ng Y-L, Spratt D, Sriskantharajah S, Gulabivala K. Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiological techniques. J Endod 2003; 29:317-332. [DOI: 10.1097 / 00004770-200305000-00001] [PMID: 12775002]

8. Sundquist G, Figdor D, Persson D, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. Oral Surg Oral Med Oral Pathol. 1998; 85:86–93. [PMID: 9474621]

9. Sundquist G, Figdor D. Life as endodontic pathogen. Ecological differences between the untreated and filled root canals. Endod Topics. 2003; 6:3–28. [DOI: 10.1111 / j.1601-1546.2003.00054.x]

10. Heid CA, Stevens J, Lival KJ, Williams PM. Real-time quantitative PCR. Genome Res 1996; 6: 986-994. [PMID: 8908518]

11. Gomes BP, Pinheiro ET, Gade-Net CR, EL Sousa, Ferraz CC, Zaia AA, et al. Microbiological examination of infected dental root canals. Oral Microbiol Immunol. 2004; 19:71–6. [PMID: 14871344]

12. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, CC Ferraz, de Souza-Filho FJ. Microbial analysis of canals of root filled teeth with periapical lesions using polymerase chain reaction J Endod 2008; 34:537–40. [DOI: 10.1016 / j.joen.2008.01.016] [PMID: 18436030]

13. Rocas IN, Jung II-Y, C-Y Lee, Siqueira JFJr. Polymerase chain reaction identification of microorganisms in previously root-filled teeth with or without periradicular lesions. J Endod 2004; 30:504–8. [PMID: 15220647]

14. Sedgley C, Nagel A, Dahlen G, C Reit, Molander A. Real-time quantitative polymerase chain reaction analysis and culture of Enterococcus faecalis in root canals. J Endod. 2006; 32:173–8. [DOI: 10.1016 / j.joen.2005.10.049] [PMID: 16427453]

15. Williams JM, Trope M, Caplan DJ, Shurgacs DC. Detection and quantification of Enterococcus faecalis by Real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and Cultivation during endodontic treatment. J Endod. 2006; 32:715–21. [DOI: 10.1016/j.joen.2006.02.031] [PMID: 16861068]

16. Ruan Y, Shen L, Zou Y, Z Qi, Yin J, Jiang J, et al. Comparative genome analysis of Prevotella intermedia strain isolated from infected root canal reveals features related to pathogenicity and adaption. BMS Genomics. 2015; 16:122–7. [DOI: 10.1186 / s12864-015-1272-3]

17. Oliviera JCM, Siqueira JF Jr, Alves GB, RJF Hirata, Andrade AFB. Detection of Porphyromonas endodontalis in infected root canals by 16S rRNA based directed polymerase chain reaction. J Endod. 2000; 26:729–32. [DOI: 10.1097 / 00004770-200012000-00016] [PMID: 11471643]

18. ET Pinheiro, BPFA Gomes Ferraz CCR, ELR Sousa, PB Texeira, Souza-Filho FJ. Microorganisms from canals of root filled teeth with periapical lesions. Int Endod J. 2003; 36:1–11. [PMID: 12656508]
Mikrobiloški status kanala korena endodonski neuspešno lečenih zuba

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UVOD
Osnovni zadatak endodonskog tretmana je da eliminiše infekciju iz kanala korena i spreči reinfekciju trodimenzionalnom hermetičkom opturacijom kanalskog sistema. Usled nemogućnosti potpune kontrole i eliminacije infekcije iz kanala korena može doći do pojave endodontskog neuspeha.

Citl ovog rada je bio da se neposredno posle uklanjanja materijala za opturaciju kod zuba sa neuspešnim endodontskim lečenjem PCR tehnikom proveri mikrobiloški status endodontskih lečenih zuba.

Metode
Mikrobiloška studija je obuhvatila 30 zuba (osam višekorenih i 22 jednokorenih) indikovanih za ponovljeni endodontski tretman. Posle dezinfekcije radnog polja i dejonturacije koja je urađena isključivo mehaničkim putem, uzorak je uzet sterilnim kanalnim instrumentom tipa pulpekstirpatora (#15) ili Hoedstrem-turpije (#15) i uz pomoć papirnih poena kojima je sušen kanal. Ependorfice su čuvane na temperaturi od -20˚C do mikrobiloške analize. Putem klasičnog PCR analizirana je zastupljenost bakterija: Enterococcus faecalis, Peptostreptococcus micros, Prevotella intermedia, Porphyromonas endodontalis i Actinobacillus actinomycetemcomitans.

Rezultati
Pozitivan bakteriološki nalaz registrovan je u 80% slučajeva, dok bakterije nisu identifikovane u 20% uzoraka. Svi negativni uzorci su uzeti iz kanala korena zuba bilo značajnijih promena u apsksnom periodoncijumu, dok je 17 od 24 kanala sa identifikovanim bakterijama pripadalo zubima sa oštećenim apsksnim periodoncijumom. Najzastupljeniji bakterijalni naklasi bio je E. faecalis, koji je identifikovan u 83,3% kanala, zatim P. intermedia sa 75% i P. micros sa zastupljenosti od 58,3%. Kod zuba sa hroničnim periapikalnim promenama najzastupljeniji je bio E. faecalis, koji je identifikovan u 94% kanala, zatim P. intermedia, koja je bila prisutna u 82,3% uzoraka. Mikroorganizmi E. faecalis i P. intermedia su registrovani kod svih pacijenata sa nekim od simptoma.

Zaključak
Prisustvo periapikalnih lezija snažnije utiče na mikrobiloški status kanala korena endodontskih lečenih zuba. Prisustvo bakterija u kanalima korena potvrđeno je u većini endodontski neuspešno lečenih zuba, a najčešće identifikovane bakterije bile su E. faecalis, zatim P. intermedia, P. micros i P. endodontalis.

Ključne reči: PCR; E. faecalis; endodontski neuspeh
zub). Kod 13 zuba uočena je adekvatna restauracija ili protetska nadoknada, sedam zuba je bilo bez ispuna duži period, a 10 zuba nije imalo odgovarajući ispun. Prisustvo simptoma u vidu bola, otoka, prisustva fistule, osjetljivosti na perkusiju ili bolovi na zagrižaj zabeleženi su kod 10 pacijenata, dok preostalih 20 nije imalo kliničke znake ni simptome.

Zdravlje apektorskog periodontijuma ocenjivano je dodeljivanjem PAI indexa, pri čemu je potpuno zdrav parodontijum (PAI 1) radiografski registrovan u šest slučajeva; PAI 2 skor (male promene u strukturi kosti koje nisu patognomonične za apikalni periodontitis) zabeležen je u sedam slučajeva; PAI 3 skor (koji podrazumeva promene u koštanoj strukturi sa dekalifikacijom karakterističnom za apikalni periodontitis) registrovan je kod 13 zuba; PAI 4 (koji predstavlja periodontitis sa jasno definisanom zonom rasvjetljenja) zabeležen je u tri slučaja, i najviši PAI 5 skor (uznapredovao periodontitis sa znacima egzacerbacije i ekspansije kosti) dodeljen je samo jednom slučaju.

Mikrobiološki postupak je uključivao najpre uklanjanje čvrstih i mekih naslaga sa zuba, eventualno postojećih restoracija i karijesnih masa i postavljanje koferdama. Dezinfekcija operativnog polja urađena je 30% rastvorom vodonik-peroksida i 2,5% rastvorom natrijum-hlorida, koji je zatim inaktivisan 5% rastvorom natrijum-tiosulfata. Dezopturacija je urađena isključivo stereoskopskim optičkim instrumentima tipa pulpekstirpatora (#15) (Tabela 2). Svi uzorci uzeti iz kanala korena zuba sa hroničnim perikutornim lezijama su bili pozitivni na prisustvo bakterija (100%).

Kada je u pitanju zastupljenost ispitivanih bakterijskih vrsta, najčešće identifikovana bakterija bila je E. faecalis (66,6%), potom E. faecium (46,6%), P. micros (46,6%) i P. endodontalis (26,6%) i A. actinomyces (10%) (Tabela 3).

Najprisutniji mikroorganizam u kanalima sa pozitivnim bakteriološkim nalazom bio je E. faecalis, koji je identifikovan u 83,3% kanala, zatim P. endodontalis sa 75% i P. micros sa zastupljenosti od 58,3% (Tabela 4). U grupi zuba sa zdravim apektorskim periodontijumom uvek bakterijske vrste (izuzev A. actinomyces, koji nije detektovan ni u jednom uzorku) bile su jednako prisutne bakterije (u polovini takvih kanala u pitanju je bio E. faecalis, koji je identifikovan u 94% kanala, zatim P. Intermedia, koja je bila prisutna u 82,3% uzoraka (Tabela 4). Kada je u pitanju broj bakterijskih vrsta prisutnih u pojedinačnom uzorku, samo u 13,3% slučajeva se radilo o monoinfekciji (u polovini takvih kanala u pitanju je bio E. faecalis), dok su najčešće (33,3% kanala) identifikovane tri bakterijske vrste u kanalu korena (E. faecalis, P. Intermedia i P. micros) (Tabela 5).

Uočeno je da uzorci uzeti iz zuba sa zdravim parodontalnim tkivima oko vrha korena pokazuju uglavnom odsustvo bakterija ili prisustvo jedne ili dve bakterijske vrste, dok je u slučaju postojanja hroničnih perikutornih promena u više od polovine uzoraka identifikovano prisustvo tri ili više bakterijskih vrsta (Tabela 6).

Korelacija između prisustva određenih simptoma posle priboljšanja parodontalnih promena i korišćenja bakterija, oštećenih kanala sa hroničnim periapikalnim lezijama, zabeleženo je u 3 iz 7 slučajeva.

Mikroorganizam E. faecalis i P. Intermedia su registrovani kod svih pacijenata sa bolovima, kod zuba osetljivih na perkusiju i bolove na zagrižaj, kao i kod polovine uzoraka uzetih iz kanaola korena zuba sa otokom i fistulom. Posebno karakteristikom je osećaj na hranjenje toka pokušavanja, koji se pojavljuje u mnogim slučajevima. Najčešće je identifikovan kod 66,6% pacijenata.
mikroorganizam bio je *A. actinomyces*, koji je identifikovan u 50% kanala sa prisutnom fistulom, kod 33,3% pacijenata sa bolovima na zagrižaj i 12,5% uzoraka uzetih iz kanala korena zuba pacijenata sa osetljivošću pri perkusiji (Tabela 7).

**DISKUSIJA**

U analizu je uključeno 30 zuba kod kojih je postojala potreba za retretmanom bez obzira na to da li je u pitanju bila protetska indikacija, slučajan nalaz ili je pacijent imao određene tegobe.

Kako bi se sprečila kontaminacija uzoraka iz kanala, sprovedeni su striktno asептични uslovi u zavojima i aktuelnim protokol rezervisanih rastvarača i lubrikanata. Mikroorganizam koji se izolovao iz kanalnih prethodno punjenih materijalnih uzoraka nije mogao da preživi sve etape endodontskog tretmana, jer su njegovi ostaci ne bi uticali na uzeti uzorak [7]. Takođe, kompletna dezopturacija je radena isključivo mehanički bez upotrebe klizača ukazivala na značaj koronarne restauracije u prevenciji reinfekcije endodontskog prostora i izneli stav da je sekundarna infekcija vazan uzročnik neuspeha endodontskog lečenja [1, 19].

Najzastupljeniji mikroorganizam iz kanala sa pozitivnim bakteriološkim nalazom bio je *E. faecalis*, zatim *P. intermedia* i *P. micros*, koje su identifikovane u 94% kanala korena zuba sa hroničnim periapikalnim lezijama (PAI 3, 4, 5), što je slično nalazima Sledgley i sar., koji su takođe PCR metodom identifikacije na 48 uzoraka dobili 90% učestalost (PAI 3, 4, 5), što je slično nalazima Sledgley i sar., koji su takođe PCR metodom identifikacije na 48 uzoraka dobili 90% učestalost (PAI 3, 4, 5). Ovo istraživanje ukazuje da su preostali mikroorganizmi u kanalima korena zuba sa hroničnim periapikalnim lezijama (PAI 3, 4, 5), što je slično nalazima Sledgley i sar., koji su takođe PCR metodom identifikacije na 48 uzoraka dobili 90% učestalost (PAI 3, 4, 5).

PCR (Polymerase Chain Reaction) savremena je, brza (potrebno je svega nekoliko sati) i jednostavna metoda za identifikaciju mikroorganizama. Izuzetno je osetljiva i visoko specifična. Teoretski je moguće dokazati prisustvo samo jedne bakterijske vrste (žive ili mrtve) u uzorku, mada se broj od 10 ćelija smatra jezičnu kvalitetu, što predstavlja ulazna vrata za sekundarnu infekciju [20]. Slično, PCR metodom identifikacije u 57,7% kanala korena zuba bez promena u apeksnom parodoncijumu krunične restauracije nisu bile zadovoljavajućeg kvaliteta, što predstavlja ulazna vrata za sekundarnu infekciju endodontskog prostora. Međutim, u ovom kontekstu je potrebna dovoljno velika učestalost i broj uzoraka u izolaciji bakterija [2].

Ovo istraživanje ukazuje da su preostali mikroorganizmi verovatno dospeli u kanal zahvaljujući neadekvatnom kručinom zaptivanju. Naime, kod 70% zuba sa zdravim apiklom, bakterije nisu imale uticaja na periradikularna tkiva. Preostali mikroorganizmi sa zastupljenošću od 58,3%.

RT-PCR-om detektovali njegovo prisustvo u uzorcima uzetim iz 57,7% kanala korena zuba bez promena u apeksnom parodoncijumu, bio je *E. faecalis*. Ovo istraživanje ukazuje da su preostali mikroorganizmi verovatno dospeli u kanal zahvaljujući neadekvatnom kručinom zaptivanju. Naime, kod 70% zuba sa zdravim apiklom, bakterije nisu imale uticaja na periradikularna tkiva. Preostali mikroorganizmi sa zastupljenošću od 58,3%.

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drugi da je na njihovom putu od usne dupljje (mikrocurenjem) kanalno punjenje blokiralo dalji prolaz ka periapeksnim tkivima.

Pacijenti koji su uzeli učešća u ovoj studiji su dolazili iz ordinacija opšte stomatološke prakse i domova zdravlja, gde je mogućnost poštovanja savremenih standarda i dobre endodontske prakse uglavnom neadekvatna, tako da je učestalost bakterijske zaostalih posle primarnog endodontskog lečenja mogla biti visoka (kako za *E. faecalis* tako i za druge mikroorganizme).

Prevotella spp i Porphyromonas spp (ranije klasifikovani kao Bacteroides spp) pripadaju grupi „crnopigmentisanih bakterija“ jer na agaru formiraju sjajne, glatke kolonije sive ili crne boje. Po novoj taksonomiji, saharolitične vrste Bacteroides spp su svrstane u rod Prevotella, a asaharolitične u rod Porphyromonas. Vrste P. intermedia, P. melaninigenica, P. denticola i P. dentalis pripadaju gram-negativnim obligatnim anaerobima. Iako imaju ograničenu sposobnost fermentacije aminokiselina i zahtevaju prisustvo hemina i menadoina za rast, mogu se uočiti u različitim delovima organizma (usna duplja, gornji respiratorni i urogenitalni trakt) [25]. Ruan i sar. su ustanovili da *Prevotella intermedia* poreklom iz usne dupljje, gornji respiratorni i urogenitalni trakt) [25]. Ruan i sar. su ustanovili da *Prevotella intermedia* poreklom iz usne dupljje predstavlja potencijalno oportunistički mikroorganizam koji se povezuje s periodontalnim obolelijima ali i s periapikalnim periodontitisom jer posjeduje adhezivnost i kompetitivnost sa okolnim mikroorganizmima [25].

Porphyromonas endodontalis je gram-negativan mikroorganizam koji se dovodi u vezu sa periodontitisom, endodontskom infekcijom i gingivitisom, i to češće sa simptomatskim nego asimptomatskim infekcijama u usnoj duplji [26].

Trecina pacijenata koji su uzeli učešća u studiji su prijavili neki od kliničkih simptoma, a najčešće registrovani simptom bila je osetljivost na perkusiju. Najčešće prisutni mikroorganizmi u uzorcima uzetih iz takvih kanala su bili *E. faecalis* i *P. intermedia* (100%), a zatim *P. micros* (50%). Gomes i sar. su takođe našli statistički značajnu povezanost *P. micros* i osetljivosti zuba na perkusiju [12], a Pinheiro i sar. su sa ovim simptomom povezali prisustvo *P. intermedia* [27].

*E. faecalis* i *P. intermedia* su registrovani kod svih pacijenata sa bolovima, osetljivošću na perkusiju i bolovima na zagrižaj, kao i kod polovine uzoraka uzetih iz kanala zuba pacijenata sa otokom i fistulom. *P. micros* je identifikovan kod 66,6% pacijenata sa bolom, a Pinheiro i sar. ovo takođe povezuju sa prisustvom bakterijskih vrsta *P. intermedia* i *P. micros* [27].

ZAKLJUČAK

Prisustvo periapikalnih lezija značajno utiče na mikrobiološki status kanala korena endodontski lečenih zuba. Prisustvo bakterija u kanalima korena potvrđeno je u većini endodontski neuspešno lečenih zuba, a najčešće identifikovane bakterije bile su *E. faecalis*, zatim *P. intermedia*, *P. micros* i *P. endodontalis*. 