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Antibacterial effect of irrigants and medications for temporary dressing on Enterococcus faecalis

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Abstract

Summary: *The main etiological factor for the development of the apical periodontitis is the microbial infection. Enterococcus faecalis is the microorganism most commonly found in asymptomatic, persistent endodontic infections. An important goal of the endodontic treatment is the removal of pathogenic microorganisms or reduction to levels compatible with the healing process.*

Objective: *The study was conducted to thoroughly investigate the benefits of the various medications used for irrigation and temporary dressing against the most common pathogen in teeth with chronic apical periodontitis, namely Enterococcus faecalis.*

Materials and methods: *The study included 61 teeth diagnosed with chronic apical periodontitis. 31 of them are subject to one-visit treatment, white the remaining 30 - a multi-visits treatment. A Microbiological examination was performed before the treatment of the root canals and immediately after their preparation. In the case of multi-visits treatment, the last sample was taken after application and response to the intracanal medicaments used as a temporary dressing. In the presence of an old root canal obturation, the first microbiological sample was taken immediately after removal of the obturation.*

Results: *A relatively large proportion of the microbiological studies revealed the presence of Enterococcus faecalis in the root canals before mechanical and chemical treatment. A relatively large proportion of the re-examination after root canal treatment did not detect the presence of pathogenic microorganisms.*

Conclusion: *In our study we prove that adequate isolation of the operative field, proper mechanical and chemical treatment, including only EDTA, sodium hypochlorite 5.25% and saline (distilled water), are sufficient to control infection and reduce microorganisms (Ent.faecalis, C .albicans, E.coli, Pseudomonas aeruginosa) in the root canal system.*

Keywords: Enterococcus faecalis, endodontic treatment, calcium hydroxide, sodium hypochlorite, microorganism

Introduction

The main etiological factor for the development of apical periodontitis is microbial infection. An important goal of endodontic treatment is the removal of pathogenic microorganisms or reduction to levels compatible with the healing process. In order to achieve optimal results from endodontic treatment, bacterial populations in the root canal must be eliminated or at least significantly reduced to levels compatible with the healing process of periapical tissues. The endodontic environment provides a selective habitat for the creation of a mixed, predominantly anaerobic flora [1]. This polymicrobial community and the environment form an ecosystem that depends on many factors, both on the part of the microorganism and on the part of the microorganisms [2]. The most common microorganisms responsible for CPP are the resistant Candida albicans and Enterococcus faecalis [3]. Their main biological and pathogenic properties are antigenicity, mitogenic activity, chemotaxis, enzymatic histolysis and activation of host cells [4]. Regardless of which of the two methods - one-visit or multi-visits work and what medications are used, they need to have the ability to affect these resistant pathogens.

Aim

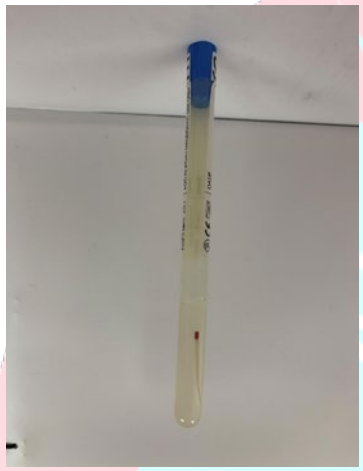
Focusing on additional microbiological identification of isolated microorganisms from the root canal system, we aim to confirm that Enterococcus faecalis is the most common pathogen in teeth with chronic apical periodontitis. The study was conducted in order to thoroughly investigate the benefits of various medications used for irrigation and temporary dressing against this resistant microorganism.

Materials and methods

The first part of our study is a survey among dentists, in order to determine how many of them apply additional microbiological research to identify microorganisms, as well as which are the isolated microorganisms. For the needs of the forthcoming study, a special anonymous questionnaire was created, which was filled in by 80 dentists from Varna and Sofia regions. The survey was conducted during scientific and educational events.

In the second part of our study all examined patients signed informed consent. The diagnosis of all 61 teeth is chronic apical periodontitis, proven by clinical and paraclinical studies. If necessary, preendodontic build up is performed. Endodontic treatment begins with isolating the operative field with a rubber dam. An endodontic cavity was prepared using a diamond cylindrical bur, an Endo Z bur (Dentsply Maillefer) and a Gates Glidden-bur. If present, the old canal filling is removed with retreatment files - Protaper Retreatment D1, D2 and D3 (Dentsply maillefer). The working length is determined by an electrometric method using an apexlocator IPexII (NSK, Japan). An initial microbiological sample is taken at this stage of treatment to prove the presence of pathogenic microorganisms in the root canals. The surface layer of dentin from the root canal was scraped with a sterile K-file. The material is taken with a sterile paper point that stays inside for 10 seconds. It is transported to the laboratory in Amies liquid transport medium (Fig. 1). The apical part of the root canal is treated manually to No.20 K-file, and the subsequent expansion of the canal is achieved by machine tooling with the help of Protaper Next X1 and X2. The treatment of the root canals start with irrigation with 2 ml. 5.25% NaOCl for 30 to 60 seconds per canal. Distilled water is used to neutralize its effect. Next step is an irrigation with 2 ml. 5.25% NaOCl using ultrasonic activation at a wavelength of 45 kHz (Ultra X, Eighteeth). A distance from the tip of the tool to the full working length must be provided within

a minimum of 5 mm. to avoid the release of irrigation solution outside the root canal into the periodontal space and the surrounding bone. This is followed by rinsing with distilled water and washing with 17% EDTA solution [5]. At the end of the preparation, all irrigations must be neutralized. Drying of the root canals with sterile paper points for Protaper Next follows. Following is the second microbiological test. The material is taken with a sterile paper point that stays inside for 10 seconds. It is transported to the laboratory in an Amies transport medium. The root canals are obturated with a thermoplastic gutta-percha using the Soft-Core Heater (CMS Dental) [6].



In the case of teeth treated by a multi-visits method, the method already described is applied at the first visit. Two microbiological samples are required before root canal treatment and immediately after preparation. The next step is to place a dry sterile swab in the cavum or calcium hydroxide. The tooth is hermetically obturated with a temporary filling material for a period of 7 days. After this period, the tooth is re-isolated with a rubber dam, opened and the swab or intracanal medication removed. The last microbiological test follows. The material is taken through a sterile paper points that stays inside for 10 seconds. It is transported to the laboratory in Amies liquid transport medium. Final irrigation is started with 5.25% NaOCl, which is activated by passive ultrasonic irrigation for 20 seconds. This is followed by rinsing with distilled water and washing with 17% EDTA solution. The final rinsing is with distilled water - 2 ml per canal [6].

Fig. 1. Amies liquid transport medium

Laboratory methods

Colombia blood agar (Fig. 2) or a chromogenic identification medium (Fig. 3) is used for the cultivation of the microorganisms. Cultivation is performed at 37° C. After 24 h, grayish colonies 1-2 mm in size were observed on the blood agar, showing an α hemolytic zone, weak β hemolysis or non-hemolytic at all. They form small lactose-positive colonies on the intestinal media.

Fig 2. Columbia blood agar medium

Fig. 3. Chromogenic identification

Method for identification of streptococci and enterococci: Serves to differentiate streptococci and enterococci from catalase-positive genera of facultative anaerobic gram-positive cocci. In a drop of distilled water on a glass slide, dilute one yoze of the test culture, then add a drop of 3% hydrogen peroxide. With a positive reaction, bubbles of varying intensity appear after about 1 minute. If blood agar colonies are used,

care should be taken not to take parts of the culture medium, as erythrocytes contain catalase, which may lead to false positives.

Fig. 4. Bile-esculin test

Esculin hydrolysis: Esculin hydrolysis (bile-esculin test) and growth in broth with 6.5% NaCl is used to identify the Enterococcus genus, which are able to hydrolyze esculin in the presence of bile. Enterococci and certain streptococci hydrolyze glucoside, esculin to esculetin and dextrose. Organisms that break down esculin molecules and use the released glucose to supply energy release esculetin into the environment. Free esculetin reacts with iron citrate in the middle to form a phenolic iron complex that is dark brown or black (Fig. 4). Citric acid enters the environment as an Indicator of esculin hydrolysis and as a result the formation of esculetin.

Conglutination and latex agglutination reaction – reagent and kit Crystal BD Crystal BD (Becton Dickinson)

The BBL Crystal system uses modified conventional, fluorogenic and chromogenic substrates to identify gram-positive bacteria. It is designed to identify frequently isolated anaerobic gram-positive bacteria. Cups are observed for color change or fluorescence resulting from the metabolic activity of microorganisms (Fig. 5). The resulting sample of 29 reactions was converted into a ten-digit profile number, which was used as a basis for identification. Samples of biochemical and enzymatic reactions for the 29 BBL Crystal GP ID substrates for a wide range of microorganisms are stored in the BBL Crystal GP ID database. The identification is obtained by comparative analysis of the reaction pattern of the test isolated substance and that of the database.

Fig. 5 Kit Crystal BD (Becton Dickinson)

Results

After analyzing the results obtained from a survey of 80 dentists, we found that only 6.25% of them apply additional microbiological testing to identify endodontic pathogens. Of the five dentists who performed biochemical identification, 80% reported that Enterococcus faecalis was isolated (Fig. 6) [6].

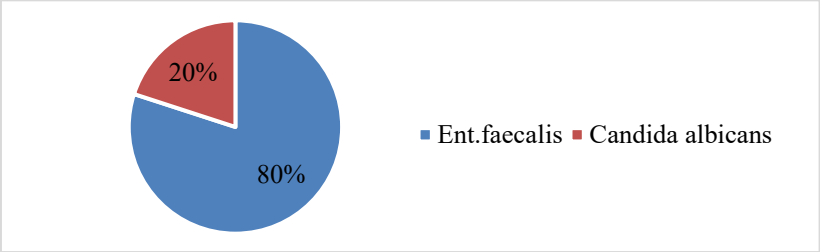


Fig. 6. Distribution of answers to question: "Which MO is most often isolated?"

From the analysis of the results obtained by the clinical laboratory, we conclude that in 19 of the samples (61.3%) taken before root canal treatment, only one MO was isolated, namely Enterococcus faecalis (Table 1) (Fig. 7). In two of the cases (6.5%) a mixed infection was found, which included Enterococcus faecalis, E. coli and Staphylococcus CNS (coagulase-negative staphylococcus). Candida albicans infection was observed in 4 (12.9%) of the samples. In one of the samples (3.2%) Pseudomonas aeruginosa was isolated in large quantities 10^5. No infection in the root canal system was observed in 5 (16.1%) of the microbiological samples taken. In 25 of the examined patients (96.1%), complete removal of microorganisms was observed after mechanical and medical treatment. Only in one patient (3.85%) persistence of some of the microorganisms was observed in the repeated microbiological examination (Fig. 8). The initial test was a polymicrobial infection caused by Enterococcus faecalis and Staphylococcus CNS (coagulase-negative staphylococcus). Repeated microbiological examination revealed the presence of only Staphylococcus CNS (coagulase-negative staphylococcus) [6].

Table. 1. Isolated microorganisms before and after chemical treatment in teeth treated by a single-visit method

	E. faecalis	Candida albicans	Pseudomonas aerugenosa	Ent.faecalis + E.coli	Ent.faecalis + Staph.CNS	Without MO
Before	19	4	1	1	1	5
After	0	0	0	0	1	30

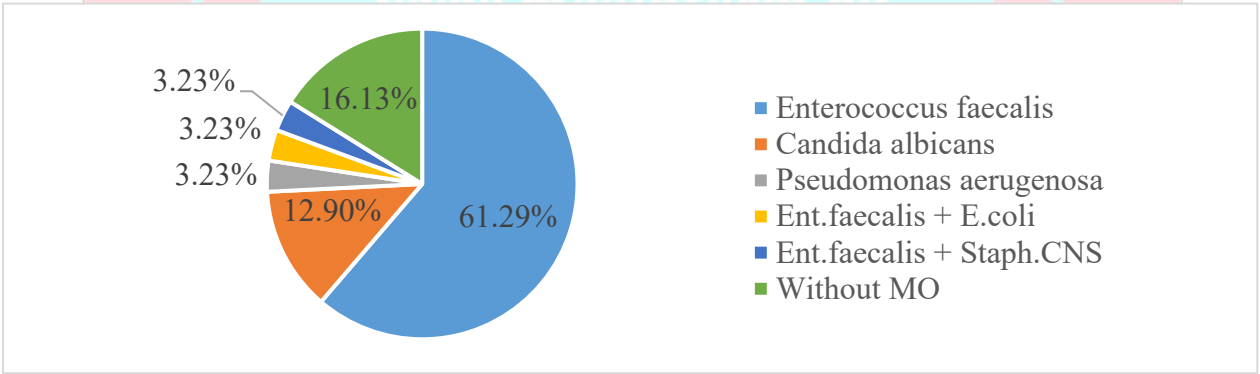


Fig. 7. Isolated microorganisms before medication treatment in teeth treated by a single-visit method

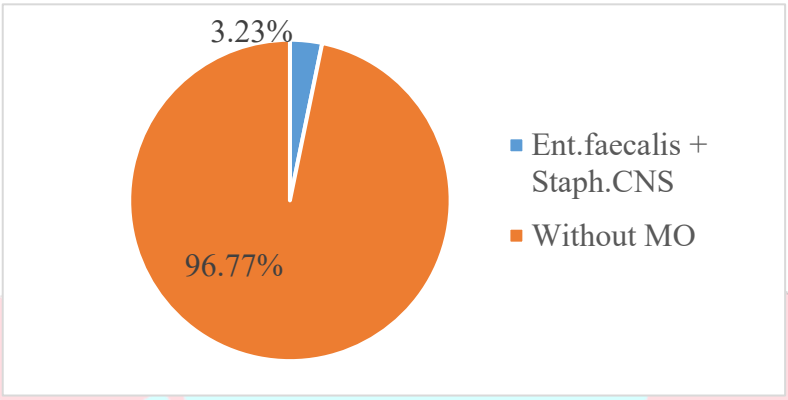


Fig. 8. Isolated microorganisms after medication treatment in teeth treated by a one-visit method

From the analysis of the results obtained by the clinical laboratory, which we conducted in 30 patients treated by a multi-visits method, we conclude that in 25 of the samples (83.3%) taken before root canal treatment, Enterococcus faecalis was isolated (Table 2). (Fig. 9). In three of the cases (10%) an infection caused by S. aureus was detected. In one (3.3%) of the samples, infection caused by M. catarrhalis was observed. In 3.3% of the microbiological samples taken, no infection was observed in the root canal system [6].

Table. 2. Isolated microorganisms before and after medication treatment in teeth treated by a multi-visits method

	Enterococcus faecalis	S.aureus	M.catarrhalis	Without MO
Before	25	3	1	1
After	0	0	0	30

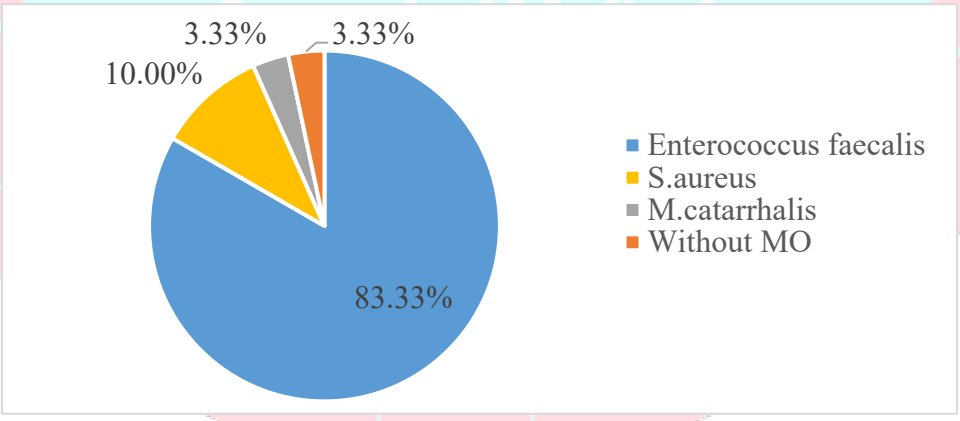


Fig. 9. Isolated microorganisms before medication treatment in teeth treated by a multi-visits method

In all examined patients (100%), complete removal of microorganisms was observed after mechanical and chemical treatment (Fig. 10). The presence of microorganisms in the root canals was not reported in any of the samples taken after the stay of the temporary dressing [6].

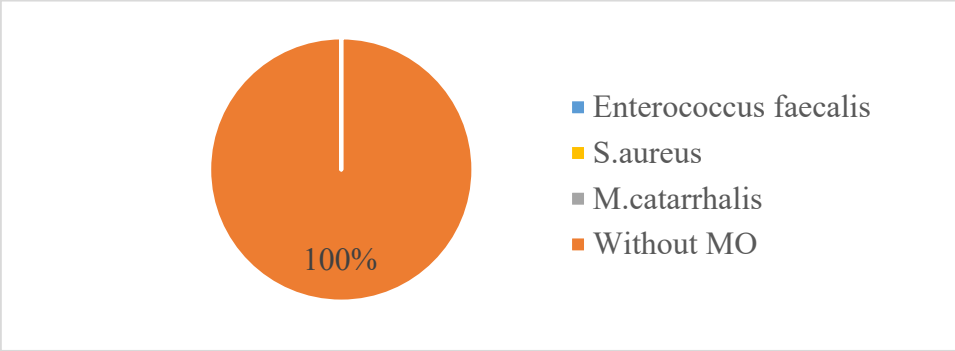


Fig. 10. Isolated microorganisms after medication treatment in teeth treated by a multi-visits method

Discussion

In our study we prove that adequate isolation of the operative field, proper mechanical and chemical treatment, including only EDTA, sodium hypochlorite 5.25% and saline (distilled water), are sufficient to control infection and reduce microorganisms (Ent.faecalis, C .albicans, E.coli, Pseudomonas aeruginosa) in the root canal system [6, 7, 8]. From a microbiological point of view, we conclude that the treatment of root canals in one visit creates favorable environmental conditions for periapical recovery, which has been confirmed by other authors [9, 10]. Our results are not confirmed by the results obtained from the study of Nair P.N.R. et al., using the same irrigation protocol but observed residual infection in 87.5% of the teeth included in the study [11].

Regarding the reduction of microorganisms and the prevention of reinfection between visits, no differences were found between the different medications for temporary application in the root canals between visits, which was not confirmed in the Ferrari PH study. et al. [12], who on repeated microbiological test established the presence of microorganisms in all examined teeth, treated by a multi-visit method without application of an intracanal dressing. The main risk with a multi-visits method of treatment is depressurization of the cavity and reinfection of the root canal system.

The results obtained from our study are confirmed by other authors [13], in whose study they proved the increased antimicrobial effect of calcium hydroxide compared to conventional drugs used. In two of her studies, Radeva E. [14, 15] confirmed the strong effect of calcium hydroxide and chlorhexidine on endodontic pathogens, which was not confirmed in the study of Zancan et al. [16]. They found that calcium hydroxide was ineffective against the elimination of E. faecalis. Paikkatt et al. [17] confirm the benefits of using this medication against the other major pathogen, Candida albicans. Contrary to these conclusions, Tonea A. et al. [18] found in their study that calcium hydroxide showed very low efficacy against Candida albicans and Enterococcus faecalis and should not be used alone as an antimicrobial agent. Supporters of this theory are other authors (Zancan R. F. 2016) [19], who prove the need for the combination of calcium hydroxide with chlorhexidine to control microbial infection, confirmed in another study [20].

Conclusion

From a microbiological point of view, we can conclude that the treatment of root canals in one visit creates favorable environmental conditions for periapical recovery. Regarding the reduction of microorganisms and prevention of reinfection between visits, no differences were found between the different medications for temporary dressing in the root canals between visits. One of the main advantages of single-visit treatment is the inability to re-infect the root canals between visits, which would be possible with multi-visits treatment.

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