ORIGINAL ARTICLE

THE ANTIMICROBIAL EFFICIENCY OF THREE ENDODONTIC IRRIGANTS ON SPECIFIC CULTURES OF ENTEROCOCCUS FAECALIS – AN IN VITRO STUDY

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ABSTRACT

Purpose. In this in vitro study the efficiency of three root canal irrigation solutions, commonly used in endodontic treatment, was evaluated, in combating a specific strain of Enterococcus faecalis, ATCC 29212. The irrigation solutions tested were 2% and 5.25% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA) and 2% chlorhexidine gluconate (CHX).
Materials and methods. Root canal preparation was performed on 20 extracted permanent single rooted human teeth. Following root canal preparation in association with irrigants, the teeth were sterilized in an autoclave and then the root canals were contaminated with E. faecalis. After 24 h of incubation, the contaminated teeth were divided into five groups of four teeth each, according to the irrigation solution used for 40 minutes of completely immersion. Dentin was collected from the root canals walls by using sterile hand files and then the dentin chips were inoculated again onto blood agar plates.
Results. On the plates seeded from the teeth immersed in 2% CHX, a significant growth of Colony Forming Units (CFU) of E. faecalis was observed. No growth on the 5.25 % NaOCl corresponding plates compartments and minimal growth on NaOCl 2.5% was recorded.
Conclusions. The power of 2% NaOCl over E. faecalis is lower than that of NaOCl 5.25%, but it is higher than CHX and EDTA solutions. NaOCl 5.25% had the best antimicrobial action.
Keywords. Endodontic treatment, Enterococcus faecalis, Columbia Blood Agar plates, Endodontic irrigants

INTRODUCTION

Culture studies have shown that the microbiota of endodontically treated teeth with apical periodontitis consists of two predominant species: Gram-negative bacteria and Enterococcus faecalis [1, 2]. The structure of bacterial communities varies from individual to individual, suggesting that certain bacterial combinations may play a role in the failure of endodontic treatment [3].

Several studies have confirmed that E. faecalis is the most common species found in endodontic treated teeth with apical periodontitis and the possibility of finding it in an endodontic treated tooth is much higher than in a primary infection [4, 5]. These results suggest that E. faecalis may be inhibited by other members of bacterial combinations present in primary infection and that it may survive under precarious conditions in an obturated root canal. Moreover, its survival rate for prolonged periods makes it resistant to NaOCl [6].

The fact that E. faecalis is the most common species found in endodontically treated teeth and that it has attributes that make it survive in such conditions has led many authors to nominate it as the main pathogen involved in treatment failure [7]. E. faecalis has various survival and virulence factors which include its ability to compete with other microorganisms, to invade the dentinal tubules and to resist nutrient deficiency [8]. Despite being found in a small percentage in the untreated root canals, it plays a major role in the etiology of resistant apical periodontitis after endodontic treatment. It is commonly encountered in the failure of endodontic treatments and it can survive in root canals as a single organism or as a major component of microbiota [9].

The pathogenicity of E. faecalis creates the need for finding effective antimicrobial irrigants. NaOCl and CHX are the most extensively investigated antimicrobial agents and they are routinely used during the endodontic treatment of root canal infection [10,11]. On the other hand, EDTA is a chelating agent used during endodontic treatment with the purpose of removing the smear layer that results from the mechanical instrumentation of the root canal. It is recommended that the chelating solutions to be used at the end of the instrumentation, followed by irrigation with NaOCl, which manages to penetrate the areas that are opened by EDTA exposure [12].

In consideration to these factors, the purpose of this study was to evaluate the antimicrobial efficiency of 2% and 5.25% NaOCl, 2% CHX and 17% EDTA against a specific strain of Enterococcus faecalis, ATCC 29212.

MATERIAL AND METHODS

The present study included 20 single rooted human teeth, recently extracted, with a single root canal, confirmed on the pre-operative X-rays. The teeth were selected following the criteria imposed by the protocols used in previous studies. In order not to dehydrate until endodontic treatment, the freshly extracted teeth were stored in 0.5% chloramine T for one week, after their prior cleaning of any traces of calculus and periodontal ligament, using an ultrasonic scaler. Prior to endodontic treatment, the dental crowns were sectioned 2 mm above the cemento-enamel junction, using a diamond

instrument in a high-speed handpiece.

The root canal content was removed using K-file instruments (Kendo, VDW GmbH, Munich, Germany) in the presence of a viscous EDTA chelating agent, from K-file # 10 to # 20, and then instrumented using the Protaper Next rotary system (Dentsply Maillefer, Baillagues, Switzerland) mounted in an X-Smart Plus (Dentsply Maillefer) endodontic motor, up to the X4 instrument (# 40), using the sequence indicated by the manufacturer, from X1-X4.

Each tooth was irrigated during the endodontic treatment with 5.25% NaOCl (Chloraxid 5.25%, Cerkamed, Poland), alternatively with 17% EDTA solution (MD-Cleanser, Meta Biomed, Korea), summing an overall irrigation time of 30 minutes for each sample. In order to increase the efficiency of root canal disinfection and for the better removal of the smear-layer and bacterial biofilm, irrigation was ultrasonically activated with an endodontic irrigation tip (IRRI S, VDW) using the Satelec Suprasson P5 Booster device (Satelec Acteon Group, France).

Figure 1.
The teeth and their individual model packed in pairs and sterilized.

order to facilitate the manipulation of the teeth during the bacteriological tests, an individual model of putty condensation silicone impression material (Zetaplus, Zhermack, Italy) was made for each tooth. This model prevented also the recontamination of the endodontic system from the root surface during the sampling of the contents from the root canal. The teeth and the corresponding silicone model were packed in pairs in sterile autoclave papers, sealed and then sterilized in an autoclave for 30 minutes at 1 atm and 135 ° C (Fig. 1).

After sterilization, to confirm the absence of bacteria in each root canal, each sample was immersed in 10 ml of Brain Heart Infusion (BHI) broth and kept in a thermostat incubator for 48 hours. The absence of bacterial contamination was demonstrated seeding the Columbia Blood Agar culture media using the inoculation loop. The Agar plates were incubated in humid environment at 37 °C for 48 hours and showed no bacterial growth.

For testing, the ATCC 29212 E. faecalis strain was seeded on a Columbia Blood Agar culture media and incubated for 24 hours in order to increase the Enterococcus culture (Fig. 2a) and to be able to prepare a bacterial suspension standardized at 2 McFarland units, the equivalent of 6x108 cells/ml and (Fig. 2b).

Using sterile transfer pipettes, 5 ml of the bacterial suspension were dripped into each tooth removed from the BHI, then the teeth were placed in the silicone individual models and incubated for 24 hours at 37oC. The success of the tooth contamination and the viability of the E. faecalis strain in the root canals were confirmed by seeding on Columbia Blood Agar culture media the dentin scraped from the root canal walls (Fig. 3).

The contaminated teeth were divided into five groups of four teeth each, according to the irrigation regimen: 2% CHX (Gluco-Chex, Cerkamed) (group A), 2% NaOCl (Chloraxid, Cerkamed) (group B), 5.25% NaOCl (Chloraxid, Cerkamed) (group C), 17% EDTA (MD-Cleanser, Meta, BioMed) (group D), and saline solution NaCl 0.9% as a control group (group E).

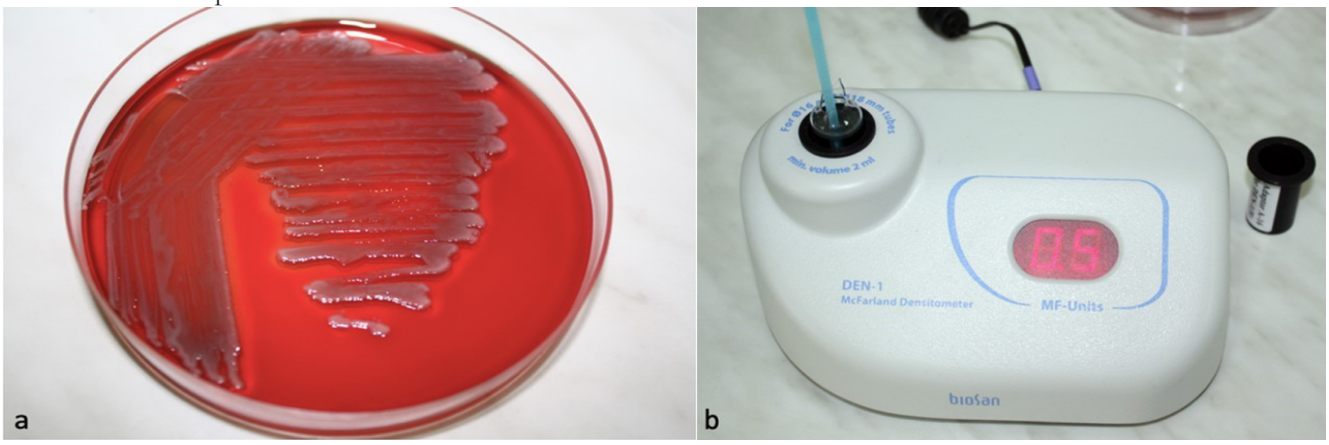

Figure 2
ATCC 29212 E. faecalis culture on a Colombia Blood Agar (a) and the solution standardization with McFarland densitometer (BioSan, Riga, Latvia) (b).

Following root canal preparation and irrigation, in The irrigation was not performed with the irrigation

syringe; instead, all the teeth were completely immersed in each solution according to the division into the above groups, in sterile containers for 40 minutes (Fig.4).

Figure 3.
The teeth seeded with E. faecalis and incubated for 24 hours

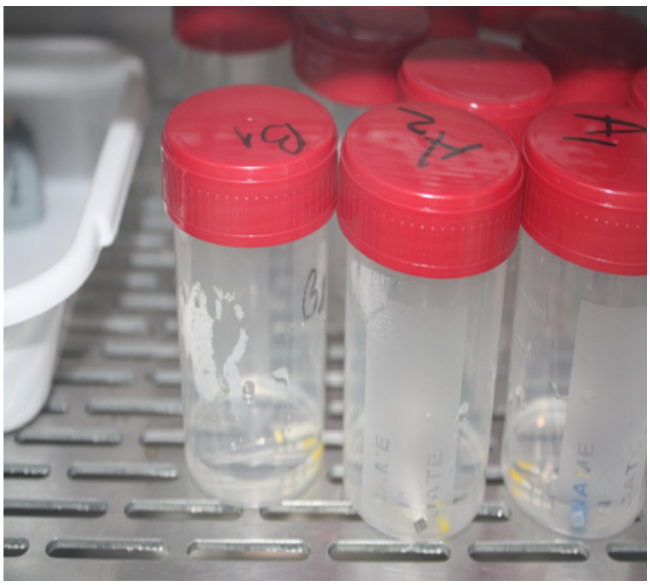

Figure 5.
The paper points and the endodontic instruments immersed in sterile saline and incubated for 24 hours at 37o C.

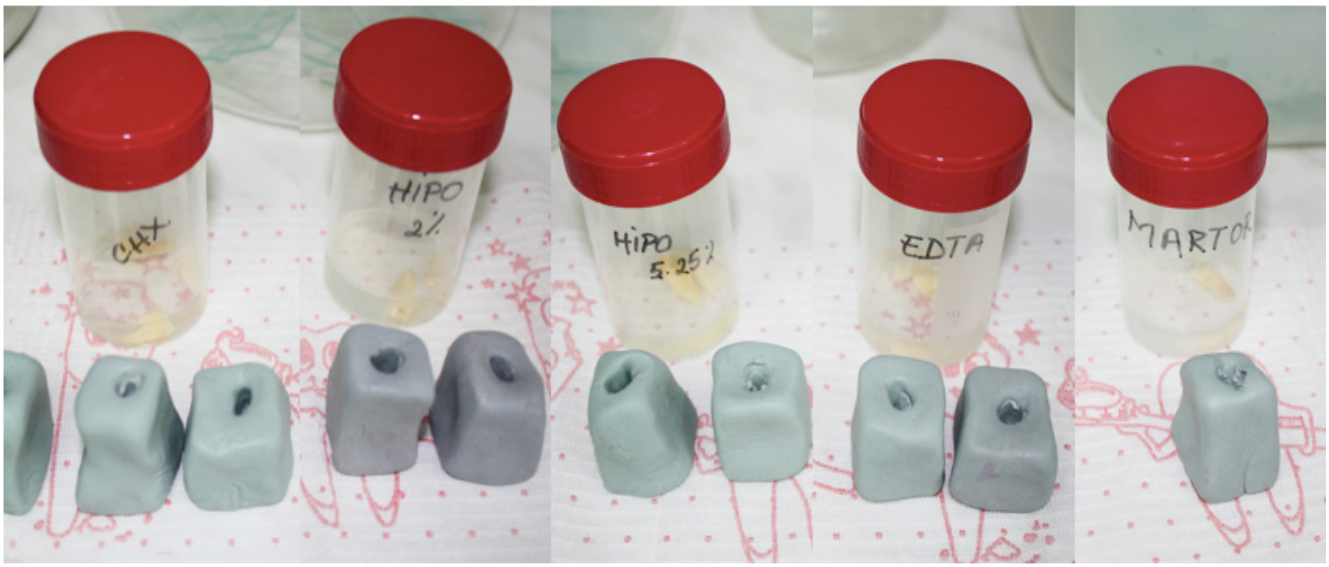

Figure 4.
Teeth divided into groups and immersed in endodontic irrigants

After immersion of the teeth, dentin was removed using ISO K-files #15 (Kendo, VDW) and sterile paper points ISO #20 (VDW) were used to colect the content from the root canal. Each K-file and each paper point that corresponds to each tooth were transferred in saline in a sterile container, separate from each other, and incubated at 37o C, for 24 hours (Fig.5). In order to see which solution was able to remove or reduce the amount of Enterococcus faecalis ATCC 29212, each sample was seeded on Columbia Blood Agar plates and incubated again in a humid environment for another 24 hours at 37o C. Each Columbia Blood Agar plate was divided into three compartments, each corresponding to the sample seeded from a specific tooth.

RESULTS

On the plates seeded from the teeth immersed in 2% CHX, a significant growth of Colony Forming Units (CFU) was observed in each compartment of the plate (A1, A2, A3) (Fig. 6a). This recording was done after 24 hours of incubation in a humid environment. So, for all chlorhexidine samples, the results were intensely positive.

The second group of plates was seeded from the teeth immersed in 2% NaOCl (compartment B1 and B2) and 5.25% NaOCl (compartment C1) (Fig. 6b). The growth of CFU in B1 and B2 compartment was significantly lower compared to the first group of plates. In the C1 compartment, no growth of CFU of E. faecalis was noticed, situation confirmed after 24 hours.

The third group of plates was seeded as follows: the C2 compartment from teeth immersed in 5.25% NaOCl; D1 and D2 from the teeth immersed in 17% EDTA solution (Fig. 6c). The results recorded 24 hours after the seeding showed no growth in the C2 compartment, while D1 and D2 were moderate positive, showing CFU growth, but lower than in the first group of plates seeded from the teeth immersed in 2% CHX.

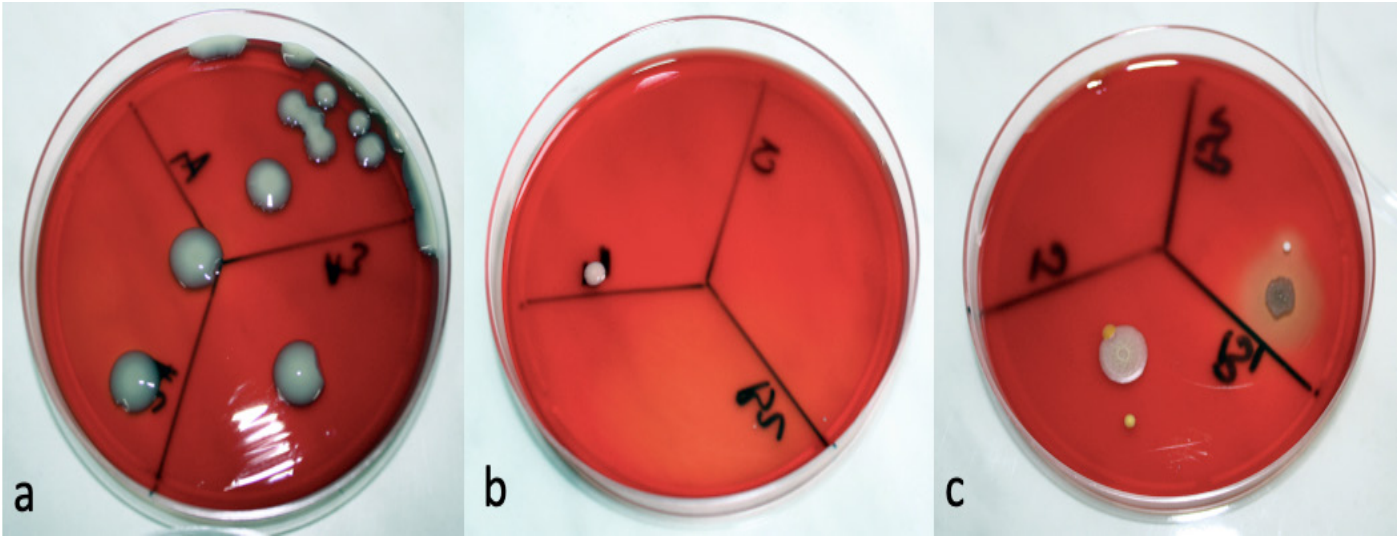

Figure 6.
Columbia Blood Agar plates seeded with the samples collected from each tooth immersed in 2% CHX (a), 2% NaOCl and 5.25% NaOCl (b), 5.25% NaOCl and 17% EDTA

DISCUSSION

For an endodontic treatment to be successful, root canal irrigation plays an important role in controlling the bacterial biofilm that is formed at the level of the endodontic system in infected teeth [13, 14, 15]. The solutions used in the present study, sodium hypochlorite, chlorhexidine and EDTA are routinely used during the endodontic treatment because they can help in the removal of the smear layer and can neutralize the root canal microbiota [16].

The ideal properties of irrigating solutions are their ability to disinfect and penetrate the dentinal tubules, to be able to provide a long-lasting antibacterial effect (substantivity), to remove the smear-layer and to dissolve the pulp tissue, to inactivate bacterial endotoxins and to be non-toxic or carcinogenic [12]. It would also be advisable not to alter the dentin structure or the sealing ability of the endodontic sealer [17]. Over time, different studies have tried to discover the most satisfactory association of the endodontic irrigation solutions that are currently used [18].

Baumgartner and Mader evaluated four different irrigation root canal regimens and according to this study no irrigant can, by itself, to dissolve the pulp, predentin and at the same time dissolve the inorganic layer of the root canal walls [19]. Instead, it has been proven that the combination between NaOCl and EDTA used alternatively during mechanical treatment is the most effective, completely removing the smear-layer from the root canal walls. The authors also demonstrated that the combination of these two irrigants has a much better antimicrobial effect than NaOCl alone [19].

For substantivity, the clinicians also use for irrigation chlorhexidine, because its efficacy results from the ability of this substance to adhere to the negatively charged surfaces of the oral bacteria, and then are gradually released from these retention areas, thus maintaining an antimicrobial activity that can last up to several hours. Only chlorhexidine and tetracycline are known to have this attribute [20].

Studies with similar results have been reported by Vianna et al. In a clinical study they evaluated the degree of microbial reduction after chemo-mechanical preparation of the root canals containing necrotic pulp using two endodontic irrigants: 5.25% NaOCl and 2% CHX gel. The bacterial load was measured in real time using polymerase chain reaction (PCR). The bacterial decrease in the NaOCl group was significantly higher than in the CHX group, probably due to the difference between the mechanisms of action of NaOCl and CHX [21].

The failure of the endodontic treatment is manifested by the symptomatic apical periodontitis, whose etiology is very complex and consists in large number of microorganisms in the root canal, but unfortunately, only a small number can be cultivated [22]. Nearly all oral infections are polymicrobial in nature and at the same time difficult to study because more than half of the microbiota cannot be cultivated on conventional culture media [23]. It has been estimated that less than 2% of the microorganisms on the surface of the earth can be cultivated [24]. On the other hand, in molecular biology, polymerase chain reaction (PCR) [25] is used to make many copies of a specific DNA segment which are exponentially amplified to generate thousands to millions of more copies. PCR is now a common and often indispensable technique but the equipment and the procedure are both expensive.

Moreover, it is difficult to find patients with this particular pathology, symptomatic apical periodontitis following an endodontic treatment, because they tend to

to resort to self-medication with antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs), which both can interfere with the apical inflammatory exudate microbiota, leading to negative or false results [26].

CONCLUSIONS

The most effective irrigant was found to be 5.25% NaOCl followed by 2% NaOCl, 17% EDTA and 2% CHX solutions.

The power of 2% NaOCl over E. faecalis is lower than that of NaOCl 5.25%, but it is higher than CHX and EDTA solutions, with only slight increases of CFU on culture media.

For the results to be more conclusive, further studies are necessary to use a larger number of teeth, as well as a quantitative analysis to evaluate the number of CFUs and their reduction with different types of irrigants; the qualitative assessment based on the direct observation of CFU growth on culture media is being subjective.

ACKNOWLEDGMENTS

The present study was developed with the technical support of the Microbiology Laboratory of the Emergency Military Hospital "Dr. Victor Popescu", Timisoara, Romania.

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