Submitted: 09 August, 2024 Accepted: 28 October, 2024 Published: 03 July, 2025 DOI:10.22514/jocpd.2025.085

ORIGINAL RESEARCH

Journal of Clinical Pediatric Dentistry

Antimicrobial activity of different irrigation solutions on *Enterococcus faecalis* in the root canal of the primary teeth—an *in vitro* comparative study

Peris Celikel[1], Banu Ceyhan[1], Murat Buyuksefil[1], Anna Levchenko[2], Fatih Sengul[1,*], Sera Simsek Derelioglu[1], Mehmet Cemal Adiguzel[2]

[1]Faculty of Dentistry, Ataturk University, 25240 Erzurum, Turkey
[2]Department of Microbiology, Faculty of Veterinary Medicine, Ataturk University, 25240 Erzurum, Turkey

***Correspondence**
fsengul@atauni.edu.tr
(Fatih Sengul)

Abstract

Background: Enterococcus faecalis is a key pathogen in persistent endodontic infections, particularly in primary teeth with complex root canal anatomy. This study aimed to compare the antimicrobial efficacy of five irrigation solutions against *E. faecalis*. **Methods**: Seventy-five extracted primary teeth were prepared and inoculated with *E. faecalis* (3×10^9 CFU/mL) then divided into five groups (n = 15): 2.5% sodium hypochlorite (NaOCl), 5% ethylenediaminetetraacetic acid (EDTA), 9% etidronic acid, 17% glycolic acid, and 0.9% physiological saline (NaCl). Root canals were irrigated, and bacterial samples were collected at 30 minutes, 24 hours, and 72 hours. Bacterial counts were analyzed using ANOVA ($p < 0.05$). **Results**: NaOCl, etidronic acid, and glycolic acid significantly reduced *E. faecalis* counts within 30 minutes. EDTA showed no immediate effect but inhibited bacterial growth by 24 hours. NaCl caused a gradual reduction in bacterial load over time ($p = 0.02$). **Conclusions**: Etidronic and glycolic acids were effective alternatives to NaOCl in disinfecting primary tooth root canals. These agents may offer biocompatible options in pediatric endodontics, but further *in vivo* studies are warranted.

Keywords

Enterococcus faecalis; Etidronic acid; Glycolic acid; Root canal irrigation; Sodium hypochlorite

1. Introduction

Microorganisms play a significant role in pulpal and periradicular diseases [1]. The main reason for the failure of endodontic treatment is the inability to remove bacteria such as *Enterococcus faecalis* (*E. faecalis*), *Streptococcus mutans* (*S. Mutans*), and *Candida albicans* from the root canal system [2]. *E. faecalis* is one of the most important bacteria in recurrent root canal infection and is among the most resistant species to treatment. Several key factors, including the ability to survive on its own, penetrate deeply into the tiny tubules in the dentin, withstand high pH levels, and in low nutrients, contribute to *E. faecalis*'s resistance to chemomechanical irrigation in the root canal [3].

The studies suggest that the root canal systems of primary teeth are more complex compared to permanent teeth [4]. Primary teeth with branching and microchannels may make it more challenging to eradicate bacteria especially *E. faecalis* from the root canals during treatment, demanding more complex procedures [5]. In addition to mechanical instrumentation, various intra-canal irrigants such as physiological saline (NaCl), sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), and ethylenediaminetetraacetic acid (EDTA) are used for bacterial elimination during root canal treatment of primary teeth. Furthermore, newly developed irrigation protocols and solutions in endodontics are promising in effectively removing bacteria from root canals [6].

Thanks to its properties such as disinfection, excellent organic tissue solvent, and anti-inflammatory action, NaOCl is the main irrigant used in endodontic treatment [7]. On the other hand, it has significant drawbacks, including a toxic property, bad smell/taste, and inability to completely remove smear layers and microorganisms, emphasizing the necessity to find new alternatives [8]. EDTA solution, which has an antimicrobial effect in removing inorganic residues such as smear layer, is also used as canal irrigant in primary teeth [9]. Etidronic acid, also known as etidronate or 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), is a chelator alternative to EDTA and citric acid and is used in combination with NaOCl [10]. Glycolic acid, which is colorless, odorless and biocompatible monomer with high solubility in water, are in the alpha hydroxy acids group containing citric acid [11, 12]. Glycolic acid offers optimal abrasion on tooth surfaces thanks to its low molecular weight and organic structure, so it has been proposed to be used as an enamel and dentin surface abrasive by replacing phosphoric acid in recent years [13]. Glycolic

acid, which is readily biodegradable [14]. It has been reported to effectively remove the smear layer from root canal dentin [15].

There are studies in the literature assessing the antimicrobial efficacy of different irrigation solutions against *E. faecalis* in primary teeth [16–19]. However, to the best of our knowledge, there is currently no report assessing the antibacterial activity of glycolic acid as an irrigation agent for the treatment of root canals in primary teeth. This study was aimed to assess the effectiveness of NaCl, NaOCl, EDTA, etidronic acid, and glycolic acid on *E. faecalis* to fill this gap in the literature. The null hypothesis of the present study is that there is no difference in the antimicrobial activity of the irrigation solutions used on *E. faecalis*.

2. Material and methods

The Local Ethics Committee of Atatürk University Faculty of Medicine (7/61, 26 October 2023) approved our study's protocol as it complies with local legislation and the principles of the Declaration of Helsinki. All parents participated in the current study signed the informed consent form.

2.1 Teeth preparation

The sample size was calculated according to a previous similar study [20]. The significant level was set at 0.05, and the statistical power of the study was set at 95%. It was estimated that 75 teeth (15 per group) were required to demonstrate an effect size (0.53).

The current *ex vivo* study included 75 primary central incisors or primary second molar palatal roots that were recently extracted due to infection or extensive decay. Teeth with resorption of more than 2/3 of the original root length and any signs of cracks or grooves in the root were excluded. The tissue residues on the teeth were removed using a Cavitron device (Cavitron® Jet SPSTM, Dentsply International Inc., York, PA, USA). Teeth were disinfected in the 2.5% sodium hypochlorite solution for 2 hours, then stored in 70% ethanol until use [21].

The dental crowns were removed with a diamond disk (Dentsply, Maillefer, Baillaigues, Switzerland), resulting in a root length of 10 mm. The working length was then determined to be 1 mm shorter than this root length. Root canals were prepared using a traditional technique with 40 K-File (Dentsply, Maillefer, USA) sizes 15 through 40 [22]. During the cleaning and shaping process, 1 mL of sterile distilled water was used after each instrument size. The canals were irrigated for 5 minutes each with 17% EDTA and 2.5% sodium hypochlorite to remove the smear layer. A final irrigation was then performed with 1 mL of 0.9% NaCl for one minute [23]. After sealing the apices with flowable composite (3 M flow Ultimate, MN, USA), the external surfaces of the tooth roots were meticulously sealed using cyanoacrylate adhesive to prevent any inadvertent leakage of the bacterial suspension. The teeth were then placed in acrylic resin (Bosworth, Neocryl, USA) blocks for easier handling during the experiment. Finally, the samples were sterilized in an autoclave at 121 °C for 15 minutes [24].

2.2 Bacterial strain and culture conditions

Frozen glycerol stocks of strain *E. faecalis* American Type Culture Collection (ATCC) 29212 in the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ataturk University (Erzurum, Türkiye) were streaked on Mueller Hinton (MH) agar plates and incubated at 37 °C for about 24 hours under aerobic condition. A fresh culture of *E. faecalis* was obtained after a sub-passage on MH agar plates for approximately 20 hours of incubation under the same conditions as described above [25]. After the incubation, bacterial strain was then diluted to 3×10^9 colony forming units per milliliter (CFU/mL) in sterile NaCl [26].

2.3 Study design

In the irrigation of each root, 5 mL of each of the five different solutions (NaOCl (260624269, Microvem, Altun Medical, Istanbul, Türkiye), EDTA (160120-1, Saver®, Prime Dental Products PVT ltd., Maharashtra, India), etidronic acid (MKCK5737, Sigma Aldrich, St Louis, MO, USA), glycolic acid (03983, Sigma Aldrich, St Louis, MO, USA), and NaCl (24474599, OSEL, Istanbul, Türkiye)) was used at the concentrations recommended for primary teeth [15, 16, 18, 27, 28]. A total of 75 primary teeth roots was randomly divided to five groups (four experimental and one control; n = 15 teeth per group), as indicated in Table 1.

A 20 μL aliquot of the suspension, with a concentration of 3×10^9 bacteria counting number (CFU/mL, was inoculated into the tooth root. The teeth were then incubated for allowing inoculation of bacteria into the teeth root at 37 °C for 30 minutes. While holding the tooth with sterile forceps, final irrigation was performed for each experimental group using 5 mL of the designated solution. The root canals were then dried using a paper point.

After 30 minutes, the root canal was irrigated with 100 μL of NaCl, and the fluid in the canal was collected for bacterial counting using a sterile pipette tip (Dragonlab, Japan). The sampled teeth were incubated at 37 °C. Similarly, sample col-

TABLE 1. Solutions used for the irrigation of the teeth root after *E. faecalis* inoculation in the current study.

Groups	Type of solution	Concentration	n	Solution volume
Group 1	Sodium hypochlorite (NaOCl)	2.5%	15	5 mL
Group 2	Ethylenediaminetetraacetic acid (EDTA)	5%	15	5 mL
Group 3	Etidronic Acid	9%	15	5 mL
Group 4	Glycolic Acid	17%	15	5 mL
Group 5 (Control)	Physiological saline (NaCl)	0.9%	15	5 mL

lection and bacterial counting procedures were meticulously performed at 24 and 72 hours [28].

For the enumeration of *E. faecalis*, a volume of 100 μL of the irrigated liquid was used. Subsequently, this volume was combined with 900 μL of physiological saline solution, and a serial dilution was performed. Aliquots of 100 μL from the appropriate dilutions were cultured on MH agar plates. These plates were then incubated at 37 °C for a duration of 24 hours. Following incubation, a random colony was meticulously selected and subsequently confirmed to be *E. faecalis* through routine biochemical tests, including Gram staining, catalase testing, oxidase testing, and Lancefield serogroup classification. The resulting bacterial count was expressed in CFU/mL [28].

2.4 Statistical analysis

Analyses were performed in SPSS version 25 (IBM Corp, Armonk, NY, USA) program. Levene's test was performed to assess the homogeneity of the time-dependent bacterial counts obtained in the current study. One-Way analysis of variance (ANOVA) was used, followed by *post-hoc* Tukey's or Dunnett's T3 tests, depending on the data distribution. A $p < 0.05$ value was considered statistically significant.

3. Results

The five different irrigation solutions were applied to the tooth roots to reduce the *E. faecalis* load (Fig. 1). NaOCl, etidronic acid, and glycolic acid showed significant bacterial inhibition within the first 30 minutes of irrigation. Surprisingly, 5% EDTA solution permitted bacterial growth during the same period ($p = 0.01$). However, as demonstrated in Fig. 1, all four solutions (NaOCl, EDTA, etidronic and glycolic acid) successfully inhibited *E. faecalis* growth at both the 24-hour and 72-hour intervals. Statistical analysis revealed varying degrees of effectiveness among these substances compared to control groups ($p < 0.001$). The use of physiological saline solution in the control group resulted in a gradual reduction in bacterial load over each timeline ($p = 0.02$). These results emphasize the importance of selecting appropriate root canal irrigants to achieve effective microbial control in endodontic procedures.

4. Discussion

The excision of necrotic pulpal tissue, dentin debris, intracanal bacteria, and the avoidance of subsequent infections are crucial in endodontic treatment [5]. Endodontic treatment of primary teeth requires some considerations. Since primary teeth have a complex root canal system due to their structure, root canal irrigation is crucial in removing microorganisms from areas where mechanical instrumentation is insufficient. However, root canal irrigation solutions currently used cannot completely remove microorganisms from the canal. Therefore, the research for an ideal irrigation solution for primary tooth root canal treatment continues [4]. Accordingly, the present study aimed to assess the effectiveness of NaCl (0.9%), EDTA (5%), NaOCl (2.5%), etidronic acid (9%), and glycolic acid (17%) in eliminating *E. faecalis* from primary tooth root

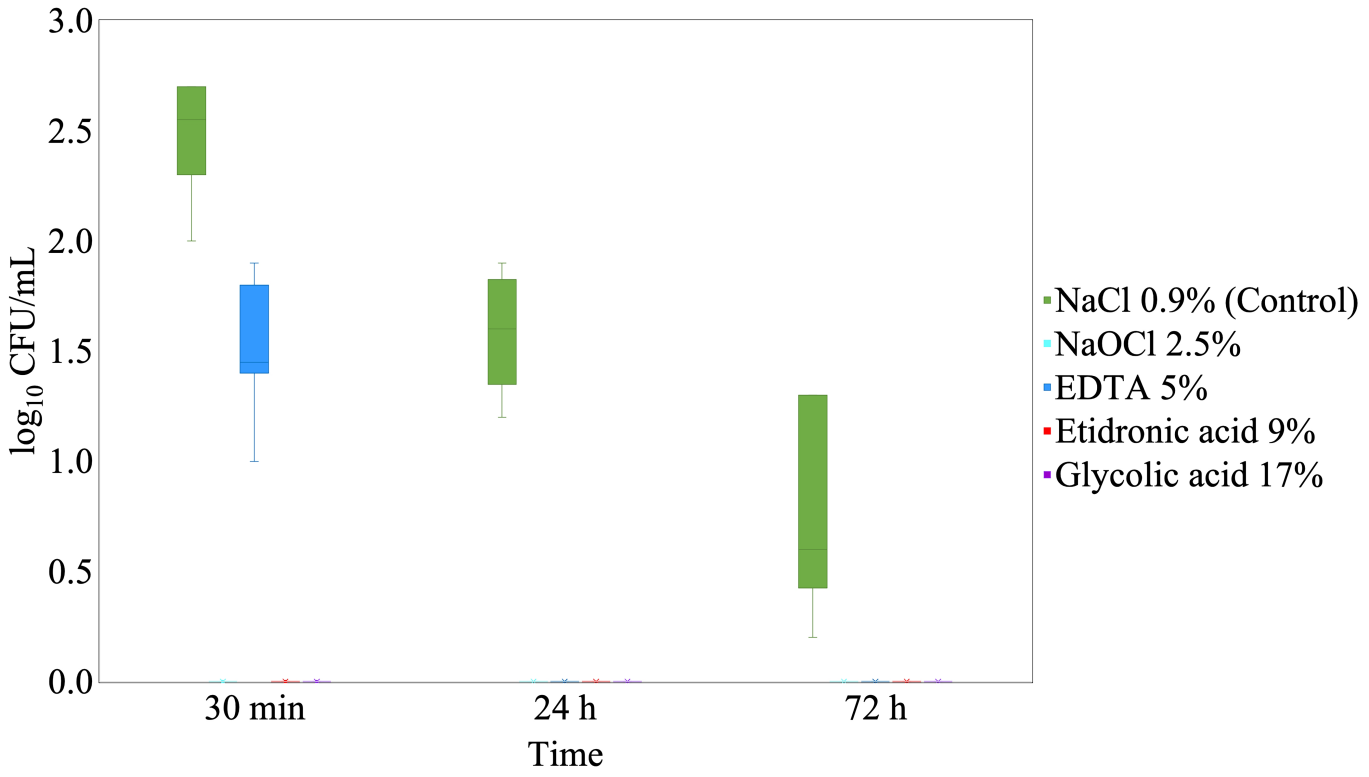

FIGURE 1. The bacteria counting number (CFU/mL) of *E. faecalis* using five different irrigation solutions for teeth root in three-time intervals. The solutions were represented with different colors on the figure. NaCl: physiological saline; NaOCl: sodium hypochlorite; EDTA: ethylenediaminetetraacetic acid.

canals. The null hypothesis was rejected; however, the results revealed that both etidronic acid and glycolic acid were promising alternatives to NaOCl, and that all three irrigants were effective.

Root canal infections are known to be associated with a wide variety of microorganisms. Studies have reported that *E. faecalis* is the most common species found in permanent tooth root canals and necrotic primary teeth [6, 29]. *E. faecalis*, an endodontic pathogen known for its resilience in challenging conditions with insufficient nutrients and its ability to survive for extended periods in treated root canals, was selected. *E. faecalis* is a Gram-positive facultative anaerobic cocci that can survive changes in pH and temperature [30]. The apices and root walls of all sampled teeth were sealed with cyanoacrylate adhesive to prevent contamination from the external surface of the tooth during the experiment in the current study.

Primary teeth have a more complex root canal system compared to permanent teeth in terms of canal structure, anatomical variations, and pulp morphology. This complexity result in bacteria within the root canal being irretrievable mechanical instruments alone, requiring the use of an effective antimicrobial irrigation agent [31]. Agents such as NaCl, NaOCl, EDTA and CHX are used in primary tooth root canal irrigation [6]. In this study, along with common solutions in the irrigation of primary teeth (NaCl, NaOCl, EDTA), the novel irrigation solutions, etidronic acid and glycolic acid were also used. However, CHX was not included in this study because recent findings have indicated that CHX is as cytotoxic as, or even more cytotoxic than, NaOCl and should not be used as a final irrigant [32].

NaCl solution is often used in primary teeth as an irrigant thanks to its isotonic nature, which minimizes harm to the tooth structure and surrounding tissues. While its antimicrobial efficacy is limited compared to other solutions, NaCl is effective at physically removing debris, tissue remnants, and microorganisms from the canal area [33]. Previous studies have reported that NaCl has limited antimicrobial activity [19, 34] and only removes debris from the root canal [35]. Similarly, the current study revealed that NaCl has a limited antimicrobial effect in the current study and should be used as a supportive agent with other antimicrobial agents.

While NaOCl, a common irrigation solution in root canal treatment, has antimicrobial and solvent properties, its use in primary canals is limited due to its unpleasant taste, potential for tissue damage, and cytotoxic effects [36]. EDTA is used to disinfect root canals, dissolving the inorganic part of the smear layer by chelating with calcium in dentin tissue [37]. While EDTA effectively dissolves the inorganic constituents of the smear layer in primary teeth, it also induces erosion of the dentin layer and exhibits a minimal antibacterial effect, hence limiting its usage [1, 38]. Therefore, it is recommended to use it in combination with NaOCl, which has both an antibacterial effect and effectively removes organic tissue [39]. In this study, while NaOCl has antibacterial effects against *E. faecalis* in the first 30 minutes, the EDTA solution was ineffective. This suggests that EDTA should not be exclusively relied upon for microbial reduction but is rather used to enhancing the efficacy of other irrigants by facilitating deeper penetration of antimicrobial solutions.

Etidronic acid is a biocompatible chelator that is used in combination with NaOCl as an alternative to EDTA or citric acid [16]. Organic acids such as EDTA or citric acid are not compatible with hypochlorite in terms of retaining active chlorine in solution. Upon the combined use of these two substances, the interaction between the calcium-EDTA complex and hypochlorite produces a precipitate. This precipitate can accumulate inside the root canal or at the canal orifice, preventing complete cleaning and disinfection of the canal. The antimicrobial properties of etidronic and glycolic acid were assessed in this study, driven by the side effects of EDTA and NaOCl. In contrast to EDTA, etidronic acid has been reported to have a decalcifying capacity that is compatible with NaOCl [40]. The use of etidronic acid as an irrigation solution in root canal treatment has been observed to reduce mechanical stress on rotary instruments [41]. Additionally, an *in vivo* study conducted on primary teeth found that 9% etidronic acid used as an irrigation solution exhibited antibacterial properties against *E. faecalis* [16]. A study by Supraja *et al.* [42] also indicated that etidronic acid may be effective in reducing bacterial load in endodontic infections.

In the literature, concentrations of etidronic acid ranging from 9% to 18% and glycolic acid from 5% to 17% have been utilized as irrigant solutions [15, 16, 27]. Glycolic acid is a biocompatible monomer of alpha hydroxy acid structure used in skin care products, dermatological treatments and tissue engineering [14]. Glycolic acid has the ability to induce collagen synthesis and fibroblast proliferation [43]. It has also been reported to exhibit similar cytotoxicity to EDTA and citric acid in cell culture and has the ability to remove the smear layer from root canal dentin. A 17% glycolic acid solution has been reported to be effective in reducing the microhardness of the superficial dentin layer and significantly facilitates biomechanical preparation under clinical conditions [15]. It has been suggested that the softening effects of glycolic acid on dentin walls may be advantageous in clinical practice due to its ability to reduce the microhardness and increase the roughness of the dentin layer [15, 44]. Gambin *et al.* [45] reported that glycolic acid exhibited better antibacterial properties against *E. faecalis* than citric acid and EDTA. The current study revealed that etidronic (9%) and glycolic acid (17%) exhibited antimicrobial activity against *E. faecalis* in as little as 30 minutes, suggesting they can be used as an alternative to EDTA and NaOCl.

A significant limitation of this study is its reliance on a simplified polymicrobial biofilm model, which fails to accurately replicate *in vivo* conditions. Conducting further studies on the efficacy of these endodontic solutions at different concentrations, using both passive and active irrigation techniques, in the presence of a more complex biofilm representative of refractory periapical infections is crucial.

5. Conclusions

NaOCl (2.5%), etidronic acid (9%) and glycolic acid (17%) were effective in eliminating *E. faecalis* from primary tooth root canals. Within the limits of the study, etidronic acid (9%) and glycolic acid (17%) as root canal irrigation materials for primary teeth could be used as alternatives to NaOCl,

which is associated with tissue and cell toxicity, irritant effects and allergic reactions. Further research is needed to assess the effectiveness of root canal irrigants that ideally disinfect primary tooth root canals *in vivo*.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

PC, BC—designed the study and carried them out. BC, MB, AL, FS—supervised the data collection, analyzed the data, interpreted the data. PC, SSD, MCA—prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol for our study was approved by the Atatürk University Faculty of Medicine Local Ethics Committee (7/61, 26 October 2023) as it complies with local legislation and the principles of the Declaration of Helsinki. All parents included in the current study signed the informed consent form.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

[1] Ozkan HB, Cobankara FK, Sayin Z, Ozer F. Evaluation of the antibacterial effects of single and combined use of different irrigation solutions against intracanal enterococcus faecalis. Acta stomatologica Croatica. 2020; 54: 250–262.

[2] Delgado RJ, Gasparoto TH, Sipert CR, Pinheiro CR, Moraes IG, Garcia RB, *et al*. Antimicrobial effects of calcium hydroxide and chlorhexidine on *Enterococcus faecalis*. Journal of Endodontics. 2010; 36: 1389–1393.

[3] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of Endodontics. 2006; 32: 93–98.

[4] Ahmed HMA. Anatomical challenges, electronic working length determination and current developments in root canal preparation of primary molar teeth. International Endodontic Journal. 2013; 46: 1011–1022.

[5] Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic Topics. 2005; 10: 77–102.

[6] Pozos-Guillen A, Garcia-Flores A, Esparza-Villalpando V, Garrocho-Rangel A. Intracanal irrigants for pulpectomy in primary teeth: a systematic review and meta-analysis. International Journal of Paediatric Dentistry. 2016; 26: 412–425.

[7] Ozlek E, Acikgoz E, Gökkaya NZ, Taşan A, Altındağ F. Histological evaluation of the debris removal efficiency of activation of sodium hypochlorite solution at different concentrations. BMC Oral Health. 2023; 23: 528.

[8] Tulsani SG, Chikkanarasaiah N, Bethur S. An *in vivo* comparison of antimicrobial efficacy of sodium hypochlorite and Biopure MTAD™ against *Enterococcus faecalis* in primary teeth: a qPCR study. Journal of Clinical Pediatric Dentistry. 2014; 39: 30–34.

[9] Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. International Endodontic Journal. 2003; 36: 810–830.

[10] Kandaswamy D, Venkateshbabu N. Root canal irrigants. Journal of Conservative Dentistry. 2010; 13: 256–264

[11] Kataoka M, Sasaki M, Hidalgo AR, Nakano M, Shimizu S. Glycolic acid production using ethylene glycol-oxidizing microorganisms. Bioscience, Biotechnology, and Biochemistry. 2001; 65: 2265–2270.

[12] Thibault PK, Wlodarczyk J, Wenck A. A double-blind randomized clinical trial on the effectiveness of a daily glycolic acid 5% formulation in the treatment of photoaging. Dermatologic Surgery. 1998; 24: 573–578.

[13] Cecchin D, Farina AP, Vidal C, Bedran-Russo AK. A novel enamel and dentin etching protocol using α-hydroxy glycolic acid: surface property, etching pattern, and bond strength studies. Operative Dentistry. 2018; 43: 101–110.

[14] Hua X, Cao R, Zhou X, Xu Y. One-step continuous/semi-continuous whole-cell catalysis production of glycolic acid by a combining bioprocess with *in-situ* cell recycling and electrodialysis. Bioresource Technology. 2019; 273: 515–520.

[15] Bello YD, Porsch HF, Farina AP, Souza MA, Silva EJNL, Bedran-Russo AK, *et al*. Glycolic acid as the final irrigant in endodontics: mechanical and cytotoxic effects. Materials Science and Engineering: C. 2019; 100: 323–329.

[16] Rapala H, Kallakuri M, Kumar GL. Comparative evaluation of antimicrobial efficacy of etidronic acid and propolis against *E. faecalis* in primary teeth: an *in vivo* study. Journal of South Asian Association of Pediatric Dentistry. 2023; 6: 51–55.

[17] Qamar S, Jayanna R, Ahuja VR. Comparative evaluation of antimicrobial efficacy of calcium hydroxide, chlorhexidine, and triple antibiotic paste in different combination forms as intracanal medicaments against *Enterococcus faecalis* in primary teeth: an *in vivo* randomized clinical trial. International Journal of Clinical Pediatric Dentistry. 2023; 16: 448–452.

[18] Gupta A, Chaudhary G, Sharma VK, Mishra A, Shalini, Seal B. Assessment of antimicrobial efficacy of aqueous ozone, green tea, and normal saline as irrigants in pulpectomy procedures of primary teeth. Journal of Advanced Medical and Dental Sciences Research. 2023; 11: 61–63.

[19] Agarwal S, Tyagi P, Deshpande A, Yadav S, Jain V, Rana KS. Comparison of antimicrobial efficacy of aqueous ozone, green tea, and normal saline as irrigants in pulpectomy procedures of primary teeth. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2020; 38: 164–170.

[20] Shingare P, Chaugule V. Comparative evaluation of antimicrobial activity of miswak, propolis, sodium hypochlorite and saline as root canal irrigants by microbial culturing and quantification in chronically exposed primary teeth. Germs. 2011; 1: 12–21.

[21] Leelapornpisid W, Wanwatanakul P, Mahatnirunkul T. Efficacy of calcium hydroxide-loaded poly(lactic-co-glycolic acid) biodegradable nanoparticles as an intracanal medicament against endodontopathogenic microorganisms in a multi-species biofilm model. Australian Endodontic Journal. 2024; 50: 89–96.

[22] Attiguppe PR, Tewani KK, Naik SV, Yavagal CM, Nadig B. Comparative evaluation of different modes of laser assisted endodontics in primary teeth: an in vitro study. Journal of Clinical and Diagnostic Research. 2017; 11: ZC124–ZC127.

[23] Zehnder M. Root canal irrigants. Journal of Endodontics. 2006; 32: 389–398.

[24] Oter B, Topçuoglu N, Tank MK, Çehreli SB. Evaluation of antibacterial

efficiency of different root canal disinfection techniques in primary teeth. Photomedicine and Laser Surgery. 2018; 36: 179–184.

[25] Celikezen FC, Sahin İ.H. Investigation of antimicrobial and antifungal effects of some boron compounds. Bitlis Eren University Journal of Science. 2023; 12: 591–595.

[26] Goulart DB, Beyi AF, Wu Z, Adiguzel MC, Schroeder A, Singh K, *et al*. Effect of danofloxacin treatment on the development of fluoroquinolone resistance in campylobacter jejuni in calves. Antibiotics. 2022; 11: 531.

[27] Karale R, Odedra KM, Srirekha A, Champa C, Shetty A, Pushpalatha S, *et al*. Effect of dentin on the antimicrobial efficacy of 3% sodium hypochlorite, 2% chlorhexidine, 17% ethylenediaminetetraacetic acid, and 18% etidronic acid on Candida albicans: an *in vitro* study. Journal of Conservative Dentistry. 2016; 19: 455–460.

[28] Moradi F, Haghgoo R. Evaluation of antimicrobial efficacy of nanosilver solution, sodium hypochlorite and normal saline in root canal irrigation of primary teeth. Contemporary Clinical Dentistry. 2018; 9: S227–S232.

[29] Cogulu D, Uzel A, Oncag O, Eronat C. PCR-based identification of selected pathogens associated with endodontic infections in deciduous and permanent teeth. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2008; 106: 443–449.

[30] Rôças IN, Siqueira JF Jr, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. Journal of Endodontics. 2004; 30: 315–320.

[31] Forghani M, Afshari E, Parisay I, Garajian R. Effect of a passive sonic irrigation system on elimination of Enterococcus faecalis from root canal systems of primary teeth, using different concentrations of sodium hypochlorite: an *in vitro* evaluation. Journal of Dental Research, Dental Clinics, Dental Prospects. 2017; 11: 177–182.

[32] Boutsioukis C, Arias-Moliz MT. Present status and future directions—irrigants and irrigation methods. International Endodontic Journal. 2022; 55: 588–612.

[33] Basir L, Abdi R, Hashemi E. Cleaning efficacy of various root canal irrigants in primary teeth: a scanning electron microscopic study. Journal of General Dentistry. 2022; 70: 29–33.

[34] Walia V, Goswami M, Mishra S, Walia N, Sahay D. Comparative evaluation of the efficacy of chlorhexidine, sodium hypochlorite, the diode laser and saline in reducing the microbial count in primary teeth root canals—an *in vivo* study. Journal of Lasers in Medical Sciences.2019; 10: 268–274.

[35] Venghat S, Hegde M, Shetty C. Irrigants used in endodontics. International Journal of Current Microbiology and Applied Sciences. 2014; 3: 126–132.

[36] Liu H, Nio S, Shen Y. Sodium hypochlorite against *Enterococcus faecalis* biofilm in dentinal tubules: effect of concentration, temperature, and exposure time. Odontology. 2024; 112: 390–398.

[37] Darrag A. Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. Tanta Dental Journal. 2014; 11: 93–99.

[38] Hariharan VS, Nandlal B, Srilatha KT. Efficacy of various root canal irrigants on removal of smear layer in the primary root canals after hand instrumentation: a scanning electron microscopy study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2010; 28: 271–277.

[39] Violich DR, Chandler NP. The smear layer in endodontics—a review. International Endodontic Journal. 2010; 43: 2–15.

[40] Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. Journal of Endodontics. 2005; 31: 817–820.

[41] Boessler C, Peters OA, Zehnder M. Impact of lubricant parameters on rotary instrument torque and force. Journal of Endodontics. 2007; 33: 280–283.

[42] Supraja AN, Arali V, Rapala H, Vemagiri CT, Kallakuri M, Kumar GLP. Evaluation of antimicrobial efficacy of etidronic acid against *Enterococcus faecalis* in primary teeth: an *in vivo* study. International Journal of Clinical Pediatric Dentistry. 2024; 17: 433–436.

[43] Bernstein EF, Lee J, Brown DB, Yu R, Van Scott E. Glycolic acid treatment increases type I collagen mRNA and hyaluronic acid content of human skin. Dermatologic Surgery. 2001; 27: 429–433.

[44] Eldeniz AU, Erdemir A, Belli S. Effect of EDTA and citric acid solutions on the microhardness and the roughness of human root canal dentin. Journal of Endodontics. 2005; 31: 107–110.

[45] Gambin DJ, Leal LO, Farina AP, Souza MA, Cecchin D. Antimicrobial activity of glycolic acid as a final irrigant solution for root canal preparation. Journal of General Dentistry. 2020; 68: 41–44.

How to cite this article: Peris Celikel, Banu Ceyhan, Murat Buyuksefil, Anna Levchenko, Fatih Sengul, Sera Simsek Derelioglu, *et al*. Antimicrobial activity of different irrigation solutions on *Enterococcus faecalis* in the root canal of the primary teeth—an *in vitro* comparative study. Journal of Clinical Pediatric Dentistry. 2025; 49(4): 148-153. doi: 10.22514/jocpd.2025.085.