# Antibacterial Efficacy of Alternative and Conventional Endodontic Irrigants on *S.mutans* and *E.faecalis*

Hilal Erdoğan[1], Pelin Ozmen[1], and Hayrun Nisa Bulbul[1]

[1]Nevsehir Haci Bektaş Veli University

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Hilal ERDOGAN[1]*, Pelin OZMEN [2], Hayrun Nisa BULBUL[3]

[1] Nevsehir Haci Bektas Veli University, Faculty of Dentistry, Department of Endodontics, Nevsehir, Turkiye. ORCID: 0000-0001-5219-4693

[2] Nevsehir Haci Bektas Veli University, Faculty of Dentistry, Department of Basic Sciences, Department of Medical Microbiology, Nevsehir, Turkiye. ORCID: 0000-0001-9496-3032

[3] Nevsehir Haci Bektas Veli University, Institute of Science and Technology, Department of Molecular Biology and Genetics, Nevsehir, Turkiye. ORCID: 0000-0001-6843-417x

* Corresponding author

**Correspondence author:**

Hilal ERDOGAN

Nevsehir Haci Bektas Veli University, Faculty of Dentistry, Department of Endodontics, 2000 Evler Mah. Zübeyde Hanım Cad. 50300, Nevşehir/Turkiye. Email:*hilalerdogan@nevsehir.edu.tr*

Tel: +90 (384) 228 10 00-22009

ORCID ID: 0000-0001-5219-4693

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**Antibacterial Efficacy of Alternative and Conventional Endodontic Irrigants on *S.mutans* and*E.faecalis***

**Objectives:** Effective removal of microorganisms from the root canal system plays an important role in the success of endodontic treatment. Sodium hypochlorite and chlorhexidine are commonly used irrigation solutions in endodontics, but due to their disadvantages, the search for alternative irrigation solutions continues. This study aimed to evaluate the in vitro antibacterial activities of alternative and conventional endodontic irrigation solutions against *Enterococcus faecalis* and *Streptococcus mutans.*

**Methods:** The following solutions were evaluated: 5.25% sodium hypochlorite (NaOCl); 2% chlorhexidine (CHX); 0.02% (200 ppm) hypochlorous acid (HOCl); 0.1% Polyhexanide (PHMB). Sterile saline was used as a negative control. Antiseptics were impregnated on disk-shaped filter papers. Then, these discs were placed first separately and then in pairs to investigate the synergistic effects of antiseptics when used alone or together, and the zone diameters were measured by the Kirby Bauer disc diffusion method.

**Results:** In *S.mutans,* 20mm inhibition zone diameter was measured for both CHX and HOCl when used alone, and the highest antimicrobial activity was observed. Approximately the same efficacy was observed in NaOCl (6mm) and PHMB (5mm). The highest efficacy was observed in CHX+HOCl (20mm) in combined use. In *E.faecalis* , the highest antimicrobial activity was observed in CHX as a 5mm inhibition zone diameter when used alone, and in CHX+HOCl (12mm) and CHX+PHMB (10mm) when used in combination.

**Conclusions:** Alternative endodontic irrigation solutions had different antimicrobial effects on test microorganisms. The use of hypochlorous acid, which is an alternative irrigation solution, together with chlorhexidine may provide microbiological advantages in clinical use.

**Keywords:** Antimicrobials, chlorhexidine, endodontics, irrigation, microbiology, sodium hypochlorite

**Antibacterial Efficacy of Alternative and Conventional Endodontic Irrigants on S.mutans and E.faecalis**

INTRODUCTION

Microorganisms are the main factor in the etiology of periapical pathology (Kakehashi et al., 1965). Therefore, endodontic treatment aims to chemomechanically minimize bacteria in the root canal system and optimally seal the root canal space to prevent recontamination (Bystrom & Sundqvist, 1981). The persistence of microorganisms after endodontic treatment causes failure, therefore irrigation solutions with bactericidal properties are used during treatment (Pace et al., 2020; Siqueira, 2001). There are many root canal irrigation solutions on the market with different contents and properties.

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are widely used in endodontic treatment and there are many studies in the literature. NaOCl has an effective organic tissue solvent and disinfection effect at high concentrations (Ayhan et al., 1999). CHX is an endodontic irrigation solution with broad spectrum antimicrobial activity, substantivity, and strong antiseptic properties. Although there are in-vitro cytotoxicity studies indicating that CHX has a higher cytotoxic effect than NaOCl (Trevino et al., 2011), there are also studies stating the opposite (Mollashahi et al., 2016). Due to the toxic effects of NaOCl and CHX on vital tissues, alternative irrigation solutions are being investigated.

Hypochlorous Acid (HOCl) has been suggested as an alternative irrigation solution to NaOCl because it provides effective cleaning of the root canal walls (Solovyeva & Dummer, 2000). There are also studies recommended for the disinfection of endoscopes, water systems of dental units, and dental impression materials (Rossi-Fedele et al., 2010). In a study, HOCl was compared with NaOCl and it was reported that they showed similar antibacterial activity on *E.faecalis,* however, HOCl showed much lower toxicity than NaOCl (Hsieh et al., 2020).

Polyhexamethylene biguanide or polyhexanide (PHMB) is an alternative to CHX (Santos et al., 2021). It has a wide spectrum of antimicrobial activity, the ability to adhere to the organic matrix, good cell and tissue tolerance, low-grade contact sensitivity risk, and wound healing stimulating effect (Arican et al., 2020; Eberlein & Assadian, 2010; Kramer et al., 2018).

In the literature, there is no study examining and comparing the antibacterial effects of conventional solutions and relatively new alternative root canal irrigation solutions on cariogenic bacteria. This study aimed to evaluate the *in-vitro* antibacterial activities of alternative and conventional endodontic irrigation solutions against*Enterococcus faecalis* and *Streptococcus mutans* when used alone or in combination.

MATERIAL and METHOD

The following commercially available irrigation solutions were evaluated: 5.25% sodium hypochlorite (NaOCl; Endosolve HP, Imicryl, Turkiye); 2% chlorhexidine (CHX; Ceraxidin-C, Imicryl, Turkiye); 0.02% (200 ppm) hypochlorous acid (HOCl; Crystalin, Natural Health Products-NHP, Turkiye); 0.1% Polyhexanide (PHMB; Actolind® w Solution, ACTO Pharma, Germany). Sterile saline was used as a negative control. Standard strains of *Streptococcus mutans* (ATCC 25175) and*Enterococcus faecalis* (ATCC 29212) were used in the study, and the strains were incubated in Columbia Agar with 5% sheep blood agar (Becton Dickinson, GmbH) at 37°C for 24-48 hours. Antiseptics were impregnated on disk-shaped filter papers. Then, these discs were placed first separately and then in pairs to investigate the synergistic effects of antiseptics when used alone or together, and the zone diameters were measured by the Kirby Bauer disc diffusion method.

RESULTS

In *S.mutans,* 20mm inhibition zone diameter was measured for both CHX and HOCl when used alone, and the highest antimicrobial activity was observed. Approximately the same efficacy was observed in NaOCl (6mm) and PHMB (5mm). The highest efficacy was observed in CHX+HOCl (20mm) in combined use. Inhibition diameters of irrigation solutions on*S.mutans* as a result of the single- and double-disc synergy method are shown in Table 1.

Tablo 1. Inhibition diameters of irrigation solutions on *S.mutans*

| **Irrigation Solution** | **Inhibition zone diameter (mm)** |
|---|---|
| CHX | 20 mm |
| NaOCl | 6 mm |
| HOCl | 20 mm |
| PHMB | 5 mm |
| CHX +NaOCl | 15 mm |
| CHX + HOCl | 20 mm |
| PHMB+ NaOCl | 6 mm |
| PHMB + CHX | 12 mm |
| PHMB + HOCl | 10 mm |
| Sterile Saline | 0 mm |

In *E.faecalis* , the highest antimicrobial activity was observed in CHX as a 5mm inhibition zone diameter when used alone, and in CHX+HOCl (12mm) and CHX+PHMB (10mm) when used in combination. Inhibition diameters of irrigation solutions on *E.faecalis* as a result of the single- and double-disc synergy method are shown in Table 2.

Tablo 2. Inhibition diameters of irrigation solutions on

*E.feacalis*

| **Irrigation Solution** | **Inhibition zone diameter (mm)** |
|---|---|
| CHX | 5 mm |
| NaOCl | 0 mm |
| HOCl | 2 mm |
| PHMB | 1 mm |

| Irrigation Solution | Inhibition zone diameter (mm) |
|---|---|
| CHX +NaOCl | 5 mm |
| CHX + HOCl | 12 mm |
| PHMB+ NaOCl | 1 mm |
| PHMB + CHX | 10 mm |
| PHMB + HOCl | 1 mm |
| Sterile Saline | 0 mm |

DISCUSSION

Microbial elimination is important in the success of endodontic treatment. *E.faecalis* is the major bacterial species isolated from root canals in endodontic failure. It has been reported that *E.faecalis* is mostly monoculture in 30-89% of teeth with post-endodontic failures (George et al., 2010). Also, it can cause life-threatening infections such as infective endocarditis (Madsen et al., 2017). *E.faecalis* is a Gram-positive, facultatively anaerobic, non-spore-forming, non-motile, cocciform bacterium (Kayaoğlu et al.). It is a microorganism resistant to harsh conditions. The virulence factors of *E.faecalis* associated with endodontic infection are lipoteichoic acid, sex pheromones, surface adhesins, extracellular superoxide, aggregation substances, lytic enzymes (gelatinase and hyaluronidase), and the cytolysin toxin, furthermore, new factors continue to be explored (Kayaoglu & Ørstavik, 2004).*E.faecalis* is related to endodontic infections, while *S.mutans* play the main role in the etiology of dental caries (Blancas et al., 2021). *S.mutans* can cause oral infections as well as induce systemic infections such as cardiovascular disorders (Lucchese, 2017). The importance of microbiological control in root canals increases when the relationship between focal oral infections and human systemic diseases is regarded. Considering all these conditions, *E.faecalis* and *S.mutans* species, which are the main factors in the pathology of oral and endodontic diseases, were used in this study.

According to the results of the current study, when used alone in *S.mutans,* the highest antimicrobial activity was observed in CHX and HOCl (20 mm inhibition zone diameter), while approximately the same activity was observed in NaOCl (6 mm) and PHMB (5 mm). In combined use, the highest efficiency was determined in CHX+HOCl (20mm). In *E.faecalis* , the highest antimicrobial activity was observed in CHX (5 mm) when used alone, while the efficacy of HOCl and PHMB was higher than NaOCl. In combination, the highest efficacy was observed in CHX+HOCl (12 mm) and CHX+PHMB (10 mm). It was observed that the antibacterial activity of HOCl and PHMB, which are alternative irrigation solutions, were higher than NaOCl. Especially in *E.faecalis* , when chlorhexidine was used in combination with alternative solutions, it was determined that the antibacterial activity increased by 2 times.

In a study evaluating the antibacterial activity of root canal irrigation solutions based on NaOCl and electrolyzed oxidizing (EO) water (HOCl), it was concluded that EO waters containing HOCl had a bactericidal effect like that of conventional NaOCl against *E.faecalis* and *S.mutans.* It has been stated that low-concentration HOCl is equally antibacterial as 1.5% and 5.25% NaOCl and considering its low toxicity, it has the potential to replace NaOCl as an alternative irrigation solution for vital pulp therapy (Hsieh et al., 2020).

HOCl, naturally produced by the human immune system to fight infection, has antimicrobial activity (Ateş; Hsieh et al., 2020). Like HOCl produced by human immunity by the myeloperoxidase-H2O2-Cl system of phagocytic cells (Pullar et al., 2000), HOCl artificially produced for disinfection also can fight invading pathogens and infections (Ateş; Hsieh et al., 2020; Lapenna & Cuccurullo, 1996). HOCl has been reported to have a toxic effect on bacteria by causing complete disruption of bacterial ATP production (Barrette et al., 1989). Compared with NaOCl in the current study, it showed greater antibacterial activity against *E.faecalis* and *S.mutans* . There are similar results in the use of HOCl as an irrigation solution for cleaning root canals and removing the smear layer in endodontic treatment (Garcia et al., 2010; Hsieh et al., 2020).

PHMB is a powerful antimicrobial solution effective against Gram-positive and Gram-negative bacteria (Messick et al., 1999), yeasts (Larkin et al., 1992), and viruses (Medvedec Mikić et al., 2018). In one study,

it was reported that 0.2% PHMB and 2.5% NaOCl solutions both successfully eliminated *E.faecalis* from mature dentin biofilm, but 0.2% CHX was not effective enough (Medvedec Mikić et al., 2018). Another study showed that 0.2% PHMB exerted significantly greater persistence on human dentin than 2% CHX (Chandki et al., 2020). PHMB is recommended as a suitable alternative to CHX as it reduces oral biofilm and has no reported side effects (Santos et al., 2021). In the current study, it was observed that 0.1% PHMB has antibacterial activity that can be an alternative to NaOCl. There is limited data in the literature on the use of PMHB as an irrigation solution in the endodontic literature, so more studies are needed on it.

This study showed that 5.25% NaOCl was not efficient enough to eliminate *E.faecalis* like the observations of other studies (Liu et al., 2010; Peciuliene et al., 2001; Portenier et al., 2003). It has been suggested that the resistance of *E.faecalis* to NaOCl may be due to its binding affinity to collagen fibers and hydroxyapatite (Chivatxaranukul et al., 2008; Kayaoglu et al., 2008). Although similar results were seen in different studies, there was no host factor such as dentin in the current study.

In this study, the most effective antimicrobial solution was 2% CHX in eliminating *E.faecalis* and *S.mutans,* and these findings are consistent with the literature (Önçağ et al., 2003; Vianna et al., 2006). Few studies have tested *E.faecalis* against disinfectants without including host factors (Kayaoğlu et al., 2008). In a study,*E.faecalis* suspensions were treated with 2% CHX for 1 hour, and negative culture was observed (Fouad & Barry, 2005). In another study, it was found to be eliminated in 1 minute with 2% CHX gel (Gomes et al., 2006). It has been reported that 0.5% CHX has a good antibacterial effect after 1 and 24 hours of application (Kayaoğlu et al., 2008).

A greater antibacterial effect can be achieved in the root canal microflora with the combination of solutions compared to the single solution, because of the synergistic and/or additive effects of irrigants with different antimicrobial activities (Ozkan et al., 2020; Sundqvist, 1992). In the current study, it was observed that the antibacterial activity of CHX in combination with HOCl and PHMB was 2 times higher than the use of CHX alone on *E.faecalis* . Since there is no study in the literature on the combined use of relatively new alternative irrigation solutions, the findings cannot be compared with other studies. In addition, NaOCl + CHX showed less antibacterial activity compared to the use of CHX with alternative solutions, like in another study (Ozkan et al., 2020). It has been stated that the reason for this may be the orange-colored precipitate formed by parachlorophenol (PCU) or chlorophenylguanidyl-1,6-diguanidyl-hexane (PCGH) when CHX and NaOCl are used together (Basrani et al., 2007; Nowicki & Sem, 2011). To prevent the interaction of different solutions, it is recommended to irrigate the root canals with saline, sterile distilled water, or alcohol between solutions, and aspirate the remaining irrigant in the canal with a needle and dry it with paper cones or perform ultrasonic activation with EDTA (Bui et al., 2008; Keles et al., 2020; Prado et al., 2013). However, in this study, the above-mentioned methods were not applied to prevent the interaction of the solutions due to the difference in the experimental design.

In the present study, the absence of host factors such as blood, serum, dentin, and collagen in the experimental design prevents the results from being reflected in the *in-vivo* environment and constitutes the limitation of the study. In this technique, the absence of dentinal tubules that protect bacteria can be advantageous for disinfectants. However, this study evaluated the protection mechanisms of bacteria against different irrigation solutions in a standard *in-vitro*environment without host factors. There are many studies in the literature with different techniques and materials. Further studies are needed on endodontic alternative irrigation solutions, such as cleaning, antimicrobial, biocompatibility, effects on tooth structure, etc.

## CONCLUSION

Within the limitations of this study, HOCl and PHMB irrigation solutions were found to have sufficient antimicrobial activity to be an alternative to conventional solutions for *S.mutans* and*E.faecalis.* It was determined that the combination of CHX and alternative irrigants increased the antibacterial activity. In particular, the combined use of HOCl with CHX may provide microbiological advantages in clinical use.

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