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Comparison of the antimicrobial effect of sodium hypochlorite, superoxidized water, and photodynamic therapy against Enterococcus faecalis in Endodontics

INVESTIGATION

Estudio comparativo del efecto antimicrobiano del hipoclorito de sodio, agua superoxidizada y terapia fotodinámica frente al Enterococcus faecalis en Endodoncia

Comparação do efeito antimicrobiano do hipoclorito de sódio, água superoxidada e terapia fotodinâmica contra Enterococcus faecalis em endodontia

Abstract

One of the main challenges in endodontics is combating Enterococcus faecalis due to its ability to resist various antimicrobial treatments and its role in the failure of endodontic procedures. E. faecalis is a gram-positive bacterium capable of forming biofilms within root canal systems, which grants it resistance to antimicrobial agents and the host's immune defenses. It is the microorganism most frequently found in secondary infections and endodontic failures. Objective: To compare the sensitivity of Enterococcus faecalis to three antimicrobial strategies (2.5% sodium hypochlorite, superoxidized water, and PDT). Method: One hundred lower human premolars were selected and instrumented with #25 K-files, then contaminated with a pure culture of E. faecalis. They were divided into four groups, each treated with a different antiseptic. Results: All three antiseptic therapies reduced the initial number of microorganisms. Sodium hypochlorite and photodynamic therapy showed similar effectiveness, while superoxidized water produced better results. Conclusions: All three alternatives are suitable for root canal sanitation, with improved outcomes observed when used in combination.

Martin Puch Damiano[1]
Ramón Álvarez-Vaz[2]
Lourdes Zaffaroni[3]
Beatriz Vilas[4]

CORRESPONDENCE
Martín Puch Damiano:
martinpuch@odon.edu.uy

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1 Prof. Adj., MSc. Departamento de Cirugía Buco maxilo facial, Sub-unidad de Farmacología y Terapéutica general y Buco dental. Facultad de Odontología, Universidad de la República.

2 Doctor en Cs Médicas, Prof.Agdo. Departamento de Salud Colectiva Facultad de Odontología, Universidad de la República.

3 Asistente Departamento de Diagnóstico en Medicina y Patología Oral, Sub-unidad Microbiología General y Buco dental. Facultad de Odontología, Universidad de la República.

4 MSC, PhD. Profesora titular Departamento Odontología Preventiva y Restauradora Sub-unidad Endodoncia. Facultad de Odontología, Universidad de la República.

Resumen

Uno de los desafíos en el campo de la endodoncia es combatir al Enterococo faecalis debido a su capacidad de resistir a diversos tratamientos antimicrobianos y su implicancia en el fracaso de los tratamientos endodónticos. Él E. Faecalis es una bacteria grampositiva que puede formar biopelículas en los sistemas de conductos radiculares, lo que le confiere resistencia a los agentes antimicrobianos y a las defensas del sistema inmunitario del hospedero. Es el microorganismo que se encuentra con más frecuencia en las infecciones secundarias y fracasos endodónticos. Objetivo: Comparar la sensibilidad del Enterococcus Faecalis a tres estrategias antimicrobianas (hipoclorito de sodio 2.5%, agua superoxidizada y PDT) Método: Se seleccionaron 100 premolares humanos inferiores, que se instrumentaron con limas tipo K # 25, luego se contaminaron con un cultivo puro de E. Faecalis. Se dividieron en 4 grupos donde se aplicaron los diferentes antisépticos. Resultados: las tres terapias antisépticas lograron reducir el número inicial de microorganismos, siendo el hipoclorito de sodio y la terapia fotodinámica similares en su respuesta y el agua superoxidizada mostró mejores resultados. Conclusiones: las tres alternativas pueden utilizarse para el saneado radicular, logrando mejores resultados las combinaciones de las mismas.

Resumo

Um dos desafios no campo da endodontia é o combate ao Enterococcus faecalis, devido à sua capacidade de resistir a diversos tratamentos antimicrobianos e ao seu envolvimento no insucesso dos tratamentos endodônticos. E. faecalis é uma bactéria gram-positiva que pode formar biofilmes nos sistemas de canais radiculares, o que lhe confere resistência aos agentes antimicrobianos e às defesas do sistema imunitário do hospedeiro. É o microrganismo mais frequentemente encontrado em infecções secundárias e insucessos endodônticos. Objetivo: Comparar a sensibilidade do Enterococcus faecalis a três estratégias antimicrobianas (hipoclorito de sódio a 2,5%, água superoxidada e PDT). Método: Foram selecionados 100 pré-molares inferiores humanos, instrumentados com limas K #25 e posteriormente contaminados com uma cultura pura de E. faecalis. Os dentes foram divididos em quatro grupos, nos quais foram aplicados os diferentes antissépticos. Resultados: As três terapias antissépticas foram eficazes na redução do número inicial de microrganismos. O hipoclorito de sódio e a terapia fotodinâmica apresentaram respostas semelhantes, enquanto a água superoxidada demonstrou melhores resultados. Conclusões: As três alternativas podem ser utilizadas na higienização radicular, com melhores resultados obtidos por meio de suas combinações.

Palabras clave: Enterococcus faecalis, hipoclorito de sodio, agua superoxidizada, fotoquimioterapia.

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Introduction and background

The oral cavity is the initial part of the digestive tract and communicates with the exterior through a septic cavity. It is colonized by the oral microbiota, which often forms microbiomes in equilibrium with the host. When microorganisms that are normally balanced within the oral cavity colonize dental structures such as root canals, periapical areas, or periodontal pockets, odontogenic infections can develop. These are characterized as polymicrobial infections.[1,2]

INFECTION IN ENDODONTICS

Knowledge of the microbiota and effective infection control within root canal systems are key determinants of the success or failure of endodontic treatment. In endodontic infections, the predominant microorganisms are facultative anaerobes and Gram-positive species. Enterococcus faecalis is particularly associated with secondary or persistent infections.[3][4] Based on the extensive literature on the subject, the prevalence of endodontic failures ranges between 5% and 10% of treatments.[5][6]

The most frequently reported factors associated with treatment failure include: microbial persistence (intra- and extraradicular), inadequate cleaning and shaping, and/or insufficient sealing of the root canal system. These factors may arise from obturations lacking proper apical sealing, leakage from clinical crown restorations, untreated canals, inadequate access cavities, perforations, false paths, instrument separation, among others.[7] Inadequate apico-coronal sealing allows tissue fluids rich in glycoproteins to infiltrate the canal, providing a substrate for residual microorganisms. This enables them to proliferate and reach sufficient numbers to generate or sustain periradicular lesions over time.[8] When coronal exposure of the root filling persists for 30 days or more, a revision of the endodontic treatment is recommended. Furthermore, since temporary cements are water-soluble and have low compressive strength, provisional restorations should be replaced with definitive ones as soon as possible.[9] Microorganisms within the root canal system may exist as planktonic cells suspended in the canal's liquid phase, or as aggregates and communities adhered to the canal walls, forming multilayered biofilms. Microorganisms living within the same community must share similar characteristics, including autopoiesis (the ability to self-organize), homeostasis (resistance to environmental changes), synergism (greater effectiveness in groups than in isolation), and the ability to respond to changes collectively rather than individually.[10]

To survive within a sealed root canal, microorganisms must resist the intracanal antiseptics used during cleaning and shaping, as well as the various topical medications, and adapt to an environment with limited nutrient availability. Bacteria located in sites such as apical deltas, isthmuses, lateral canals, irregularities, and dentinal tubules often evade endodontic antiseptic procedures, and the nutrient supply to these bacteria is likely to remain unchanged after treatment. Infection will occur if the bacteria (along with their toxins, particularly endotoxins) are pathogenic, reach a sufficient number, and access the periradicular tissues, where they can induce or perpetuate periradicular lesions.[8] The Enterococcus genus comprises species that are morphologically similar to Streptococcus. In clinical endodontics, the most frequently isolated species are Enterococcus faecalis (80–90%) and Enterococcus faecium (5–10%). They possess microbial factors that contribute to both intraradicular and extraradicular infections.[11]

Siqueira and Roças, and Sedgley et al., using the Polymerase Chain Reaction (PCR) technique, observed prevalences of E. faecalis of 77% and 79.5%, respectively. They also found that the presence of E. faecalis was more frequent in secondary infections (89.6%) than in primary infections (67.5%), with statistically significant differences.[12]

CONTROL OF INTRARADICULAR INFECTION

As the host's defense mechanisms weaken (also taking immune status into account), resistance to the entry of microorganisms into the necrotic pulp space decreases. Opportunistic microorganisms, favored by the low oxygen levels, invade, proliferate, and accumulate within the root canal system. These microbial communities are able to survive thanks to residual pulp tissue and periodontal exudate. As a result, microbial groups in necrotic teeth and in teeth with failed endodontic treatment are distributed throughout the root canal lumen, with those accessing tissue fluids primarily located in the apical zone.[13] Obligate anaerobes are more easily eradicated during root canal treatment. In contrast, facultative bacteria—such as non-mutans Streptococci, Enterococci, and Lactobacilli—once established, are more likely to survive chemomechanical instrumentation and root canal medication.[13] The control of intraradicular infection in endodontics is achieved through a combination of mechanical and chemical methods. Regarding chemical methods, irrigants penetrate the dentinal tubules and are essential for reducing the microbial load, among other applications.[14] However, with respect to mechanical methods, a significant percentage of the root canal surface remains untouched during shaping, regardless of the instruments used. These uninstrumented areas may harbor and protect microorganisms from root canal disinfection protocols.[15]

SODIUM HYPOCHLORITE

Based on the controlled laboratory studies of Koch and Pasteur, hypochlorite gained increasing acceptance as an antiseptic and disinfectant by the late 19th century.[15]

Among its advantages, sodium hypochlorite solutions are low-cost, readily available, and have a good shelf life, provided that manufacturers' specifications are followed.[13]

In water, sodium hypochlorite ionizes into sodium (Na) and hypochlorite ion (ClO^-). At pH values between 4 and 7, the chlorine ion exists as hypochlorous acid (HClO), whereas at pH values above 9, ClO^- predominates. HClO has a stronger antibacterial effect than ClO^- due to its ability to disrupt oxidative phosphorylation and other membrane-associated processes. HClO also exerts a rapid inhibitory effect on mitochondrial function and bacterial DNA synthesis. In addition to its antibacterial properties, sodium hypochlorite can dissolve pulp remnants and the organic component of dentin (i.e., nonspecific proteolytic action). It also has the capacity to partially neutralize necrotic tissue and any residual antigenic or microbial components within the root canal system, as well as to eliminate all pulp debris and predentin from uninstrumented surfaces.[20, 21] Sodium hypochlorite solution (NaOCl) is the irrigant generally used due to its organolytic, deodorizing, bleaching, and microbicidal properties.(13) However, several studies have shown that total elimination of microorganisms is not achieved consistently under current protocols. Sodium hypochlorite also has cytotoxic effects, which represents another reason for the ongoing search for alternative irrigants.(22) In our setting, sodium hypochlorite at a 2.5% concentration is accepted for use as a root canal irrigant.(15)

SUPEROXIDIZED WATER

Another irrigant used is superoxidized water. It has been shown to effectively clean the walls of the root canal system.(23) The superoxidized water solution (SOW) is a hypochlorous acid solution with a free chlorine concentration of 250 mg/L at the factory outlet (250 parts per million) and a pH of 6 (±1). In addition to this component, other chlorine-reactive compounds are present (ozone, sodium hypochlorite, chlorine, etc. <0.02%). Superoxidized water is obtained by electrolyzing sodium chloride with purified water, producing a disinfectant or antiseptic from two basic materials—salt and electricity—resulting in a final product (water) that is environmentally friendly. Hydrochloric acid and chlorine are the main products in these waters, at concentrations that vary depending on the manufacturer: between 144 and 350 mg/L. The antiseptic is generated on site by passing the saline solution over 9-amp titanium electrodes. The pH of this water ranges from 5 to 6.5 and it has a redox potential greater than 950 mV. These solutions have been shown to be neither toxic to tissues nor corrosive to equipment.(24)(25)

PHOTODYNAMIC THERAPY (PDT)

Innovative techniques for controlling polymicrobial infections have been explored in order to improve sanitation and further reduce microorganisms at the root canal level. These involve alternative methods combining irrigating agents with biophotonics procedures, such as photodynamic therapy (PDT).(26) Photodynamic therapy is a two-step treatment involving the application and retention of a photosensitizing substance (PS) in the target tissues/microorganisms. Depending on the predominant microbial species and the presence of competitors (exudate, bleeding, etc.), a waiting period—called the pre-irradiation time—is determined.(27) After this period, the excess PS is removed and a light source with a wavelength resonant with the selected PS is applied. The most extensively studied technique uses a phenothiazine dye, such as methylene blue, as the photosensitizer, and visible red light as the source. It can be applied using laser equipment with a wavelength of 660–680 nm, allowing the light beam to be directed precisely at the target (inside the canal system up to the apical zone, fistulous tract, etc.).(28) After irradiation, the methylene blue molecules absorb the transmitted electrons and remain in an excited orbit, i.e., a higher energy state. The resulting photochemical reactions stimulate the formation of reactive oxygen species (ROS). These are diverse, with singlet oxygen being one of the most abundant and metastable. ROS are highly cytotoxic due to their oxidative nature, as they interact with microbial membrane biomolecules or DNA, ultimately triggering programmed cell death.(28) The antimicrobial activity of PDT is effective against bacteria, viruses, and fungi, is non-irritating, and does not lead to bacterial resistance.(29)

Methodology

SELECTION AND PREPARATION OF TEETH

The inclusion criteria were: lower premolars with root canal lengths between 10 and 15 mm, a curvature angle of up to 25°, and a curvature radius between 5 and 8 mm. The sample will be random and stratified.

CLEANING AND SHAPING

Each canal was irrigated with 2 ml of sodium hypochlorite solution, after which the working length was established using a #10 K-file. Exploration was performed with pre-curved stainless steel #10 K-type instruments (Antaeos, VDW GmbH, Munich, Germany) up to the working length in each root canal. All specimens were shaped using Reciproc® 25 files (VDW GmbH, Munich, Germany). Next, two layers of nail polish were applied to the external surface to prevent bacterial microleakage through lateral canals or other discontinuities in the cement. To complete the preparation process, all teeth were sterilized in an autoclave cycle (Cristófoli Vitale, Brazil).

CONTAMINATION WITH ENTEROCOCCUS FAECALIS

The study followed the methodology described by Kuştarci et al. (2009).(30)

The experimental phase was carried out in the Microbiology Laboratory of theFacultad de Odontología, Udelar. The dental specimens were enumerated and divided into four groups of 25 each, using a simple random sampling technique, and were then contaminated.

A pure culture of E. faecalis from the strain bank of the Microbiology Laboratory of theFacultad de Odontología, Udelar, was used. The identity of the strain was confirmed using the MALDI-TOF technique from the Clinical Laboratory Department of the Hospital de Clínicas. The strain was cultured in brain heart infusion (BHI) broth for contamination of the root canals. McFarland standard number 0.5 was used.

A 10-µl aliquot of the bacterial culture was transferred into the lumen of the shaped canals using a sterile micropipette. The specimens were then immediately incubated for 24 hours at 37°C.

EXPERIMENTAL GROUPS

GROUP 1 Sodium hypochlorite 2.5%

Canals were initially irrigated with 1 ml of sterile saline solution (ICU-VITA Lab., Montevideo, Uruguay). Subsequently, the root canals were irrigated with 2 ml of 2.5% sodium hypochlorite solution (Lab. Leduc, Montevideo, Uruguay). Irrigants were delivered into the canals using a 5 mL disposable plastic syringe (Ultradent Products Inc., South Jordan, UT, USA) and a NaviTip needle (Ultradent Products Inc., South Jordan, UT, USA). The operator applied just enough pressure on the syringe to achieve a flow rate of 2 ml of irrigant per minute. The solution remained in the root canals for 10 minutes.

GROUP 2 Superoxidized water

Canals were initially irrigated with 1 ml of sterile saline solution (ICU-VITA Lab., Montevideo, Uruguay), followed by irrigation with 2 ml of a superoxidized water solution, Xtericold® (EMARLAND S.A. Laboratory), using the same procedure.

GROUP 3 Photodynamic therapy

Canals were initially irrigated with 1 ml of sterile saline solution (ICU-VITA Lab., Montevideo, Uruguay). As the photosensitizer, 2 ml of 0.01% methylene blue (Chimiolux 10, DMC, Brazil) was used and delivered up to the apical region using a NaviTip needle (Ultradent Products Inc., South Jordan, UT, USA). The pre-irradiation time was set at 3 minutes. The canals were then dried with Reciproc® 25 paper cones (VDW GmbH, Munich, Germany). Irradiation was carried out using a Therapy device (DMC, Brazil) at 100 mW, with a wavelength of 660 nm and an energy output of 9 Joules (J). A PDT light guide (DMC, Brazil) with a 200 µm fiber diameter was inserted into the device. The fiber was applied in a spiral motion, beginning 1 mm from the apex and progressing coronally for 90 seconds.

GROUP 4 Control group

Canals were irrigated with 2 ml of sterile saline solution (ICU-VITA Lab., Montevideo, Uruguay), delivered using a 5 mL disposable plastic syringe (Ultradent Products Inc., South Jordan, UT, USA) and a NaviTip needle (Ultradent Products Inc., South Jordan, UT, USA).

BACTERIAL EVALUATION

This was performed at two time points. The first was performed after the incubation period. A sterile Reciproc® 25 paper cone (VDW GmbH, Munich, Germany) was placed in each root canal for 1 minute to collect bacteria and confirm contamination. The paper cones were then transferred to autoclaved Eppendorf tubes (Biologix®, Biriden, Montevideo, Uruguay) containing 2 mL of BHI broth. The tubes were vortexed for 2 minutes, and 10 µL of the resulting suspension was inoculated onto one half of TSA plates, which were previously divided in two. After the treatments were applied in each group, the experimental root canals were irrigated with 1 ml of sterile saline solution. Sterile Reciproc® 25 paper cones (VDW GmbH, Munich, Germany) were again placed in the canals for 1 minute and then transferred to Eppendorf tubes containing 2 mL of BHI broth. The tubes were vortexed for 2 minutes, and 10 µL of the suspension was inoculated onto the second half of the TSA plates described above. TSA plates were incubated at 37°C for 24 hours. Bacterial colonies were counted, and results were expressed as absolute values.

STATISTICAL ANALYSIS

The analysis was conducted on 200 bacteriological cultures of Enterococcus (2 per tooth) of Enterococcus faecalis. Four treatments (sodium hypochlorite, superoxidized water, PDT, and saline) were applied to 50 seedings each. Based on the Colony Forming Units (CFUs) before and after irrigation for each treatment, the percentage reduction was calculated. Descriptive analysis included univariate methods (median and interquartile range) and bivariate methods (box and line plots). Due to the asymmetric distribution of percentage reduction values, non-parametric tests were used to compare the effectiveness of the four treatments (Kruskal-Wallis test).[31] Multiple comparisons were then performed using the Nemenyi test,[32] indicating statistically similar medians with identical letters and statistically different medians with different letters. P-values were obtained via random permutations. All statistical analyses were performed using R software (R Core Team, 2023).[33] Statistical significance was set at 5%.

Results

There were 50 CFU measurements for each of the four treatments. The percentage reduction had a median of 87.3% (ratio = 0.127), with an interquartile range of 43.3%. **Figure 1** shows the distribution of the CFU ratio before and after irrigation.

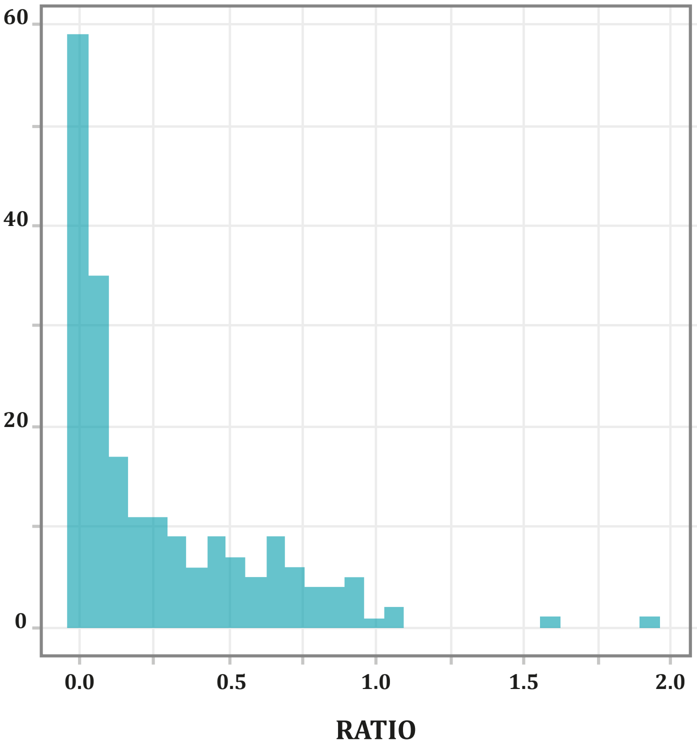


Figure 1 CFU ratio distribution before and after irrigation.

Figure 2 shows that serum had the lowest reduction percentages. In contrast, hypochlorite and PDT appeared to produce similar reductions. Ultimately, the SOW treatment yielded the highest reduction levels.

Table 1 presents the median reduction for each treatment, along with comparisons using the Kruskal–Wallis test. Complementarily, the letters accompanying the medians correspond to the multiple comparisons performed using the Nemenyi test.

TABLE 1

Efficacy of treatments on CFU reduction.

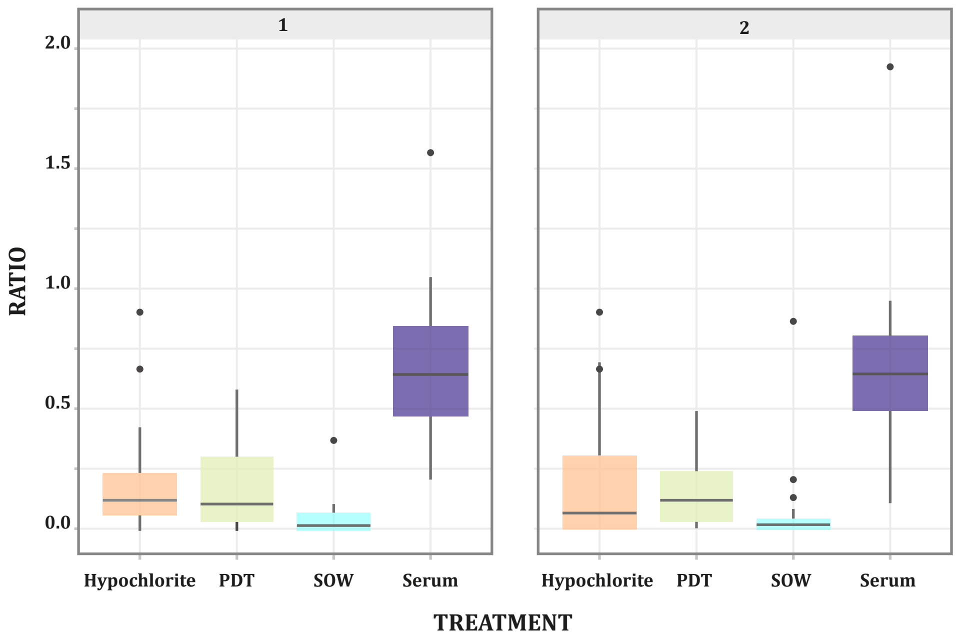
TREATMENT	OVERALL		1.ST REP	2.ND REP
Hypochlorite	9%	a	12%	8%
PDT	13%	a	11%	13%
SOW	2%	b	2%	2%
Serum	64%	c	65%	64%
KW	<0,001		<0,001	<0,001

A significant Kruskal–Wallis test value was obtained both overall and when stratifying by replicate. Based on the six p-values from the multiple comparisons (Nemenyi test), there was no significant evidence to distinguish the reduction achieved by hypochlorite from that of PDT. However, serum showed the lowest efficacy, while SOW was the most effective treatment.

Discussion

Enterococcus faecalis is a resistant pathogen commonly used as a microbiological marker in in vitro studies due to its ability to colonize root canals, form biofilms, and survive under extreme conditions. Its persistence

Figure 2 Distribution of the ratio between CFU counts before and after irrigation by replicate.



Enterococcus faecalis is a resistant pathogen commonly used as a microbiological marker in in vitro studies due to its ability to colonize root canals, form biofilms, and survive under extreme conditions. Its persistence is one of the main causes of endodontic treatment failure.[34]

Various concentrations of sodium hypochlorite have proven effective against E. faecalis. Siqueira et al. reported that a 2.5% NaOCl solution has antibacterial efficacy comparable to 5%, particularly when larger volumes are used, combined with other irrigants, or agitated to enhance its efficacy.[35] Studies by Shahram et al. showed that combining 2.5% sodium hypochlorite with a diode laser was more effective in eliminating E. faecalis than using sodium hypochlorite alone.[36]

PDT, which involves the use of light in combination with photosensitizers such as methylene blue and toluidine blue, has shown efficacy in bacterial elimination. However, its effectiveness varies depending on the concentration of the photosensitizer and the light parameters used.[37] Souza et al. found that PDT with methylene blue or toluidine blue did not produce a significant additional effect when 2.5% sodium hypochlorite was used.[38] Nair et al. reported that PDT is less effective at eliminating E. faecalis in curved root canals due to the presence of biofilms, which hinder the penetration of both the photosensitizer and the light.[39]

Research on the use of SOW as an irrigant in endodontics is limited, but some studies have demonstrated its efficacy. Zan et al. found that SOW has an antibacterial effect comparable to 5.25% sodium hypochlorite when used for 3 to 5 minutes.[40] In another study, Rossi-Fedele et al. reported that the efficacy of SOW is reduced in the presence of organic contamination but remains promising in clinical situations with lower bacterial loads.[23] Sohrab Tour Savadkouhi et al. concluded that although sodium hypochlorite eliminated 100% of E. faecalis CFU/mL, SOW achieved a 90% reduction, making it a less toxic option for endodontic irrigation.[41]

The results indicate that combining sodium hypochlorite with PDT offers greater efficacy in reducing E. faecalis. Although PDT alone is not sufficient to completely eliminate microorganisms, it serves as a promising adjuvant method. Regarding SOW, studies suggest that it may be an effective and less toxic irrigant, although its efficacy may vary depending on the bacterial load and the presence of organic contaminants.

A study by Mozayeni et al. found that PDT with methylene blue, combined with NaOCl, produced a significant reduction in CFU/mL of E. faecalis, confirming that the combined therapy is more effective than individual methods.[42]

When comparing these results with those reported by other authors, a similar conclusion can be drawn: sodium hypochlorite and PDT significantly reduced the bacterial load and, consequently, the CFUs, although they did not achieve complete elimination. However, SOW yielded the best outcome and clinical impact, as it significantly reduced CFUs, as reported by Rossi-Fedele et al.

The comparison of our results with those of Savadkouhi et al. is contradictory, as a better response was obtained with SOW than with sodium hypochlorite.

Conclusions

E. faecalis is sensitive to the three antiseptics evaluated. When comparing SOW with PDT and sodium hypochlorite solution, it can be stated that SOW exhibited superior antimicrobial activity compared to the other two antiseptics, which showed similar efficacy.

These treatments have been shown to reduce CFUs. Considering clinical application, it may be suggested that their effect would be enhanced if used in combination.

Additionally, it is worth highlighting the limited evidence available regarding SOW. Therefore, new lines of research could be developed to evaluate its efficacy against other microorganisms involved in odontogenic infections, potentially offering an alternative to sodium hypochlorite while reducing its associated adverse effects.

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Data availability

Data are available in a Google Drive folder, which contains the table with the 200 results, Ethics Committee approval, and bacterial strain validation via MALDI-TOF.

Conflict of interest declaration

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Authorship contribution and collaboration statement

NAME AND LAST NAME	ACADEMIC COLLABORATION													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Martin Puch Damiano	x			x	x	x	x	x	x	x			x	x
Ramón Álvarez-Vaz			x					x		x			x	
Lourdes Zaffaroni							x		x				x	
Beatriz Vilas	x			x		x		x	x	x		x	x	x

1. Administración del proyecto
2. Adquisición de fondos
3. Análisis formal
4. Conceptualización
5. Curaduría de datos
6. Escritura - revisión y edición
7. Investigación
8. Metodología
9. Recursos
10. Redacción - borrador original
11. Software
12. Supervisión
13. Validación
14. Visualización

This ex vivo experimental study was conducted in the microbiology laboratory of the Facultad de Odontología at UDELAR.

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