

ORIGINAL ARTICLE

The influence of chemical irrigants on the condition of the root canal biofilm in case of periodontitis treatment

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ABSTRACT

Aim: To evaluate and compare the antimicrobial efficacy of commonly used endodontic irrigation solutions against bacterial and fungal isolates recovered from infected root canals in patients with apical periodontitis, in order to determine their relative effectiveness against clinically relevant endodontic microorganisms.

Materials and Methods: The strains of opportunistic microorganisms representing the optionally anaerobic transient microflora of the oral cavity were used in the study. 11 chemical solutions used to eliminate microorganisms in the root canal, were applied in the microbiological study. Microbial cultures were isolated from the infected root canal and identified on the basis of morphological, cultural properties and such biochemical microtests as "STAPHYtest 16", "STREPTOtest 16" (Lachema, Czech Republic) and test systems VITEK 2 GP and VITEK 2 YST (bioMérieux, France) using VITEK 2 Compact analyzer.

Results: Comparing the effect of irrigation solutions on *S.aureus* MSSA bacteria, such irrigant as *Chlorhexedin* 2% (*Endohex*) provided the most positive result *Hypochlorite* 5.25% (*Cerkamed*) provided a positive effect on *C.ablicans* as well constituting 88.82 ± 1.65 in comparison with *Chlorhexedine* 2% (29.22 ± 0). Citric acid 40% (*Cerkamed*) was the best to eliminated *S.gordonii* (27.28 ± 0.35). However, *Maleic acid* 6% showed the least effect on it (6.55 ± 0.45).

Hypochlorite 5.25% (*Cerkamed*), *Hypochlorite* 6% *Chloran* showed the best influence on the *Candida* group, while *Citric acid* 40% showed the lowest influence.

Conclusions: The conducted microbiological studies showed a diverse effect of irrigation solutions on groups of different microorganisms causing periodontitis. The obtained results provide an opportunity to select differentially irrigation solutions for the elimination of microorganisms detected in the root canals. A positive result of the elimination of irrigation solutions on microorganisms causing periodontitis is observed.

KEY WORDS: apical periodontitis, irrigation solutions, microbial biofilm

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INTRODUCTION

The etiological basis of apical periodontitis is a bacterial infection of the root canal system. Despite significant advances in modern dentistry, this disease remains one of the most prevalent worldwide. Nowadays apical periodontitis is considered to be a inflammatory response of the body to the tooth pulp destruction and the root canal infection [1,2]. According to a systematic review and meta-analysis conducted by C.S. Tiburcio-Machado et al. (2019) [3], the global prevalence of apical periodontitis among the adult population is 52%, increasing to 62% in individuals over the age of 60.

However, mechanical instrumentation alone is insufficient due to the complex anatomy of root canals, which leaves 35–40% of the canal walls untouched. In addition, preparation procedures result in the formation of a smear layer that blocks dentinal tubules and creates a physical barrier, hindering the penetration of antiseptic agents into deeply located biofilms [4].

Therefore, chemical irrigation represents a critically important stage of endodontic treatment. Sodium hy-

pochlorite (NaOCl) remains the "gold standard" due to its ability to dissolve organic tissues and its pronounced antimicrobial activity. At the same time, its cytotoxicity and inability to remove the inorganic component of the smear layer necessitate the use of chelating agents such as EDTA, citric acid, or maleic acid [5].

Chlorhexidine (CHX) is considered a less aggressive alternative due to its high substantivity and long-lasting antimicrobial effect, although it lacks proteolytic activity and the ability to remove the smear layer. Although a wide range of irrigating solutions is available on the modern market, the selection of an optimal protocol is complicated by the lack of high-quality comparative data on their effectiveness against clinically significant pathogens [5].

AIM

The aim of this study was to evaluate and compare the antimicrobial efficacy of commonly used endodontic irrigation solutions against bacterial and fungal isolates

recovered from infected root canals in patients with apical periodontitis, in order to determine their relative effectiveness against clinically relevant endodontic microorganisms.

MATERIALS AND METHODS

The study protocol was approved by the Bioethics Committee of Ivano-Frankivsk National Medical University. Written informed consent was obtained from all patients prior to inclusion in the study. Clinical material for microbiological analysis was collected from infected root canals of 30 patients diagnosed with apical periodontitis or pulpitis. To ensure the accuracy and reliability of microbiological results, patients who had received systemic or local antibiotic therapy within the previous 30 days were excluded.

RESEARCH HYPOTHESIS

It was hypothesized that different chemical irrigation solutions used in endodontic treatment would demonstrate significantly different antimicrobial efficacy against clinically relevant microorganisms isolated from infected root canals in patients with apical periodontitis. In particular, sodium hypochlorite solutions with higher active concentration were expected to exhibit superior broad-spectrum antimicrobial activity due to their pronounced oxidizing and tissue-dissolving properties. At the same time, it was assumed that modified hypochlorite formulations containing surfactants might provide enhanced penetration into dentinal tubules and irregular root canal structures, thereby improving their antifungal effectiveness.

It was further hypothesized that chlorhexidine-based solutions would demonstrate strong antibacterial activity because of their well-known substantivity, but would remain limited as primary irrigants due to the absence of organic tissue dissolution. In contrast, chelating agents and organic acids, including EDTA, citric acid, and maleic acid, were considered primarily adjunctive components of irrigation protocols, contributing mainly to smear layer removal, canal wall conditioning, and facilitation of antimicrobial penetration rather than acting as potent standalone disinfectants.

Thus, the study was based on the assumption that the antimicrobial effectiveness of endodontic irrigation depends not only on the chemical identity of the solution, but also on its concentration, physicochemical modification, and functional role within the overall irrigation protocol.

MICROBIAL STRAINS

The antimicrobial activity of the irrigation solutions was studied against eight bacterial strains: α -hemolytic

streptococci of the *mitis* group (*Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus gordonii*), β -hemolytic streptococci (*Streptococcus pyogenes*, group A; *Streptococcus dysgalactiae* ssp. *equisimilis*, group G), staphylococci (methicillin-susceptible *Staphylococcus aureus* (MSSA); methicillin-resistant *S. aureus* (MRSA) with associated resistance to fluoroquinolones, macrolides, tetracyclines, and aminoglycosides; methicillin-susceptible *Staphylococcus epidermidis*), as well as five yeast-like strains of *Candida* spp. (*C. albicans*, *C. tropicalis*, *C. lipolytica*, *C. lusitaniae*, and *C. kefyr*).

Microbial cultures were isolated from infected root canals using sterile Teflon brushes, which were placed in tubes containing 1.0 ml of sterile physiological saline and thoroughly suspended for 5 minutes using a Vortex Nest mixer at 3000 rpm. The resulting microbial suspension was plated with a calibrated bacteriological loop (5 ml inoculation dose) using Gold's sectoral streaking method onto nutrient media: blood agar (for isolation and differentiation of streptococci and staphylococci), Endo medium (for enterobacteria), and Sabouraud agar (for yeasts). The cultures were incubated for 24 hours at 37°C under aerobic conditions, with streptococci additionally incubated in a CO₂-enriched atmosphere.

Identification of the isolated pure cultures was performed based on morphological, cultural, and biochemical characteristics using STREPTOtest 16, STAPHYtest 16, and the VITEK 2 GP and VITEK 2 YST test systems (bioMérieux, France) on the VITEK 2 Compact analyzer.

Tested irrigation solutions. Ten irrigation solutions were included in the study and are presented in Table 1 in the same sequence and under the same commercial names as used throughout the manuscript.

EXPERIMENTAL DESIGN

The study was designed as a controlled in vitro comparative microbiological experiment. Each tested irrigation solution was evaluated against all isolated microbial strains under identical laboratory conditions. Sterile isotonic saline solution (0.9% NaCl) served as the negative control. For each microbial strain, all experiments were performed in triplicate to ensure reproducibility, analytical reliability, and statistical validity of the obtained results.

ASSESSMENT OF ANTIMICROBIAL ACTIVITY

Antimicrobial activity of the irrigation solutions was evaluated using the agar-gel diffusion micromethod. Molten agar (30 ml) was poured into Petri dishes placed on a strictly horizontal surface. After solidification, wells with a diameter of 4.0 mm were punched. The agar sur-

Table 1. Antimicrobial activity of irrigation solutions on root canal microorganisms (growth inhibition zone diameters, mm)

Microbial strains	Control Saline solution 0.9%	Sodium hypochlorite 2% Cerkamed (Poland)	Sodium hypochlorite 5.25% Cerkamed (Poland)	Sodium hypochlorite 5.25% Extra + Deterg. Cerkamed (Poland)	Sodium hypochlorite 6% (Ukraine)	Sodium hypochlorite 6% Chemoplus Chloran (Poland)	Chlorhexidine 2% Cerkamed (Poland)	Chlorhexidine 2% Endohex (Ukraine)	EDTA 17% + Benzalkonium bromide (0.1%) Endo-Solution (Poland)	Maleic acid 6% (Ukraine)	Citric acid 40% Cerkamed Poland)
<i>S. aureus</i> MSSA	0	22.22±1.50 _a	25.63±0.14 _a	22.65±0.09 _a	19.33±0.26 _a	22.22±0.49 _a	28.06±0.25 _a	30.95±0.51 _a	24.17±0.46 _a	8.97±0.53 _{ag}	28.88±0.96 _{af}
<i>S. aureus</i> MRSA	0	29.73±1.65 _{ab}	47.45±3.24 _a	39.20±1.31 _a	24.17±0.69 _a	43.80±1.93 _a	25.69±0.18 _a	28.07±0.24 _a	22.26±0.52 _{ae}	8.41±0.87 _{ag}	28.36±0.55 _{afg}
<i>S. epidermidis</i>	0	41.34±1.12 _{ab}	49.94±1.12 _a	42.24±1.68 _a	28.86±1.47 _a	43.16±1.52 _a	31.65±0.37 _a	36.01±0.44 _a	25.91±0.53 _{ae}	9.25±0.87 _{ag}	34.14±0.66 _{afg}
<i>S. pyogenes</i> (β Str A)	0	13.54±0.34 _{ab}	17.13±0.26 _a	14.66±1.12 _a	11.68±0.69 _a	14.89±0.42 _a	20.17±0.36 _a	23.7±0.35 _a	18.27±0.24 _a	7.44±0.95 _g	25.74±0.43 _{af}
<i>S. dysgalactiae</i> ssp. <i>equisimilis</i> (β Str G)	0	7.76±0.47 _{ab}	15.06±0.28 _a	14.09±0.29 _a	11.16±0.33 _a	8.71±0.30 _a	19.38±0.12 _a	21.79±0.78 _a	16.65±0.28 _a	6.32±0.53 _g	26.09±0.37 _{af}
<i>S. gordonii</i>	0	12.30±0.21 _{ab}	16.11±0.32 _a	11.80±0.49 _a	11.91±0.77 _a	15.57±0.45 _a	20.37±0.95 _a	22.94±0.15 _a	17.63±0.44 _a	6.55±0.45 _g	27.28±0.35 _{af}
<i>S. oralis</i>	0	15.92±0.61 _{ab}	14.56±0.70 _a	13.59±0.57 _a	11.87±1.17 _a	15.59±0.45 _a	23.72±0.39 _a	24.75±0.28 _a	21.39±0.36 _{at}	7.13±0.12 _a	26.84±0.61 _{af}
<i>S. sanguinis</i>	0	13.25±1.11 _a	14.99±0.73 _a	15.29±0.42 _a	11.36±0.28 _a	14.10±0.16 _a	21.42±2.20 _a	24.51±0.72 _a	22.90±0.76 _{ae}	10.53±0.90 _a	20.98±0.23 _{af}
<i>C. albicans</i>	0	55.46±1.12 _{ab}	88.82±1.64 _a	74.60±0.63 _{ac}	41.96±0.71 _a	79.80±0.51 _a	29.22±0.64 _{ad}	30.25±0.97 _{ad}	35.46±0.48 _{ae}	0 _g	0 _g
<i>C. tropicalis</i>	0	43.77±1.05 _a	51.86±1.11 _a	40.50±1.29 _a	15.88±0.65 _{ab}	48.86±1.19 _a	26.73±0.41 _{ad}	25.64±0.62 _{ad}	28.63±0.47 _{ae}	0 _g	0 _g
<i>C. lipolytica</i>	0	37.84±0.74 _a	54.47±1.63 _a	35.10±1.06 _{ac}	18.75±0.20 _{ab}	48.46±0.49 _a	28.89±0.85 _{ad}	29.21±0.41 _{ad}	39.08±0.50 _a	0 _g	0 _g
<i>C. lusitanae</i>	0	56.64±1.05 _{ab}	84.76±1.25 _a	59.64±1.10 _{ac}	22.4±0.37 _{ab}	59.00±1.41 _a	26.59±0.41 _{ad}	29.23±0.32 _{ad}	32.03±0.42 _{ae}	0 _g	0 _g
<i>C. kefyr</i>	0	36.57±0.68 _a	44.99±0.54 _a	35.44±0.97 _a	14.61±0.20 _{ab}	39.50±0.39 _a	27.70±0.71 _{ad}	29.01±0.30 _{ad}	37.48±1.04 _a	0 _g	6.89±0.63 _g

Note. Superscript letters indicate statistically significant differences ($p < 0.05$, Dunn's post hoc test with Holm correction) when compared as follows:

- a – any irrigant vs. negative control (saline solution);
- b – Sodium hypochlorite 2% vs. Sodium hypochlorite 5.25% (Cerkamed);
- c – Sodium hypochlorite 5.25% with detergent vs. Sodium hypochlorite 5.25% without detergent;
- d – Chlorhexidine 2% vs. any sodium hypochlorite solution;
- e – EDTA 17% + benzalkonium bromide 0.1% (Endo-Solution) vs. Sodium hypochlorite 5.25%;
- f – Citric acid 40% (Cerkamed) vs. Maleic acid 6%;
- g – Citric acid 40% (Cerkamed) or Maleic acid 6% vs. Sodium hypochlorite 5.25%.

Source: compiled by the authors of this study

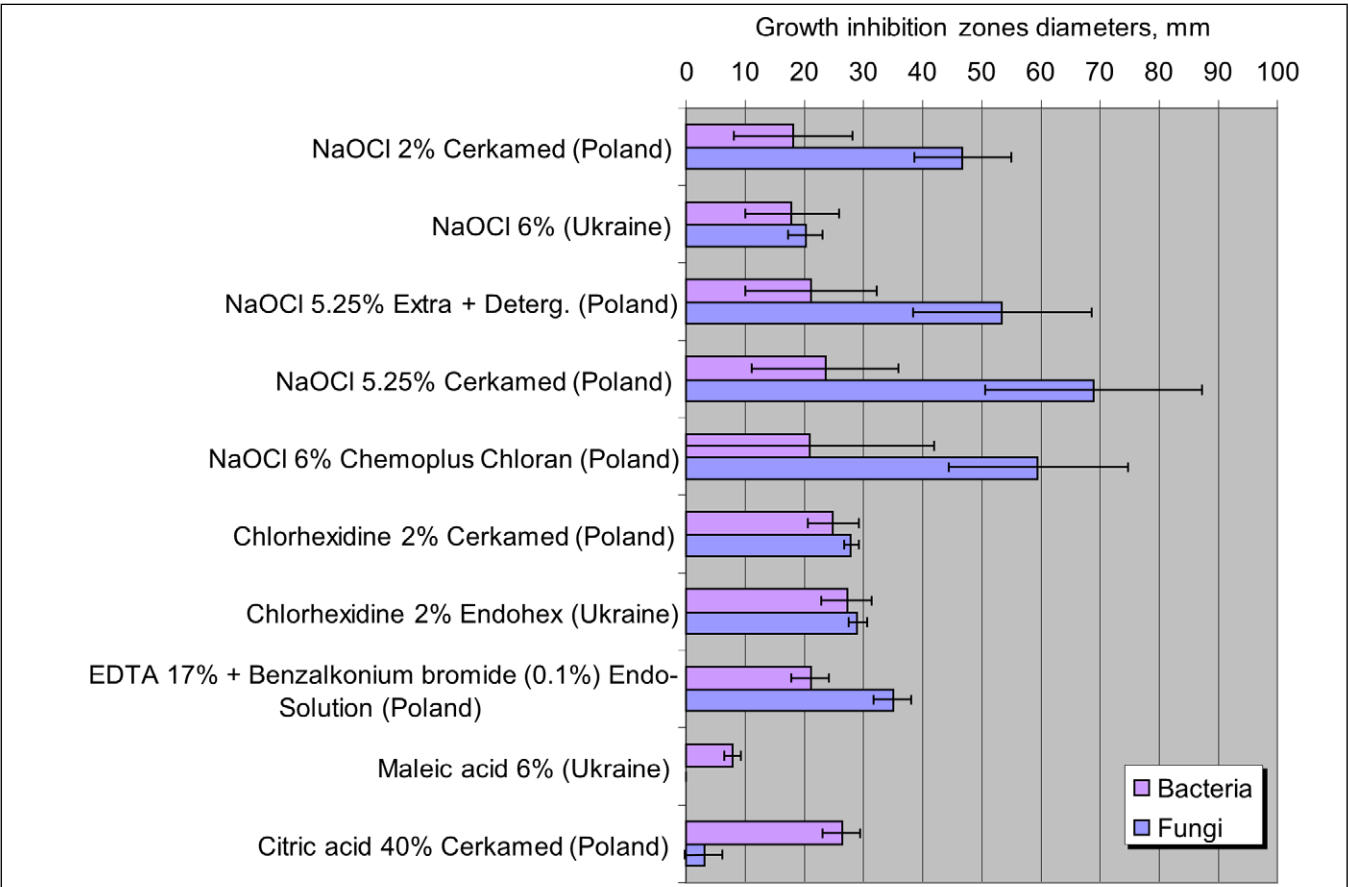


Fig. 1. Average values of growth inhibition zones diameters for each irrigation solution for all strains of bacteria and fungi separately
Picture taken by the authors

face was evenly inoculated with microbial suspensions at a concentration of 1×10^7 CFU/ml. A volume of 20 ml of each tested irrigant and sterile saline (control) was added to the wells. The diameters of the growth inhibition zones were measured after 24 hours of incubation at 37°C for bacteria and after 48 hours for fungi. Digital images of the plates were obtained and analyzed using ImageTool 2.0 software (UTHSCSA ImageTool 2.0, University of Texas Health Science Center at San Antonio, 1995–1996). For each microbial strain, all experiments were performed in triplicate to ensure reproducibility and analytical reliability of the obtained results.

STATISTICAL ANALYSIS

Growth inhibition zones are presented as mean ± standard deviation. Preliminary statistical assessment revealed deviations from normal distribution (Shapiro–Wilk test) and heterogeneity of variances (Levene’s and Bartlett’s tests). Therefore, two complementary statistical approaches were applied: the non-parametric Kruskal–Wallis test with Dunn’s post hoc analysis (Holm correction) and Welch’s ANOVA with Tukey’s HSD post hoc test. All statistical analyses were performed in R ver-

sion 4.5.1 using appropriate packages for robust ANOVA and multiple-comparison correction. A significance level of $p < 0.05$ was considered statistically significant.

AI STATEMENT

The author states that no AI tools were used in writing this article.

RESULTS

The study included 13 microorganisms capable of causing periodontitis and ten irrigation solutions intended to eliminate pathogenic biofilm within the root canal system. The tested irrigants represented various concentrations of sodium hypochlorite (2%, 5.25%, 5.25% Extra with detergent, and 6%), chlorhexidine 2% (from two manufacturers), EDTA 17% with benzalkonium bromide (0.1%), maleic acid 6%, and citric acid 40%.

Comparison of the efficacy of key irrigation agents: Sodium hypochlorite 5.25% — the clinically recognized standard; Chlorhexidine 2% — an alternative with a broad antimicrobial spectrum; Chlorhexidine 2% Endohex — a commercial CHX modification; comparison

with other hypochlorite solutions, acids, and chelating agents.

Growth inhibition zone diameters for all tested strains (mean $\pm$ standard deviation) are presented in Table 1. Pairwise comparisons between irrigation solutions were performed using the Kruskal–Wallis test followed by Dunn's post hoc analysis with Holm correction.

The results demonstrated that sodium hypochlorite 5.25% (Cerkamed) exhibited statistically equivalent antimicrobial activity to sodium hypochlorite 6% and sodium hypochlorite 6% (Chemoplus Chloran) across all tested strains ($p = 0.999$), indicating no significant difference in efficacy between these concentrations or commercial formulations. In contrast, sodium hypochlorite 2% (Cerkamed) showed significantly lower antimicrobial activity compared with 5.25% NaOCl in 11 out of 13 strains, including *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus* spp., and *Candida* spp. ($p = 0.0286$). Only α -hemolytic *Streptococcus sanguinis* did not reach statistical significance ($p = 0.0571$), although a trend toward decreased efficacy was observed.

The detergent-enhanced formulation, sodium hypochlorite 5.25% Extra + Detergent (Cerkamed), demonstrated a significant increase in antifungal activity against *Candida albicans*, *C. lipolytica*, and *C. lusitaniae* ($p = 0.0286$) compared with the corresponding formulation without detergent. These findings suggest that the addition of a detergent to sodium hypochlorite may enhance its antifungal activity, especially against *Candida* species. The trend observed across bacterial strains, while not statistically significant, may warrant further investigation with larger sample sizes to clarify potential synergistic or antagonistic effects. For all other microorganisms tested, including *Staphylococcus aureus*, *Streptococcus* spp., and other *Candida* species, no significant differences were observed ($p = 0.999$), indicating comparable efficacy between the two formulations.

The both tested Chlorhexidine 2% solutions (Cerkamed and Endohex) exhibited statistically equivalent antimicrobial activity to all sodium hypochlorite-based formulations (all $p = 0.999$). Similarly, no significant differences were observed between Chlorhexidine 2% and Chlorhexidine 2% Endohex for any strain. Simultaneously Chlorhexidine 2% demonstrated significantly greater antimicrobial activity compared to Maleic acid 6%, EDTA 17% with benzalkonium bromide (0.1%), and Citric acid 40% for the majority of tested strains. These differences were particularly pronounced for all *Candida* species (p -values were consistently below 0.05).

Maleic acid 6% exhibited significantly lower antimicrobial activity compared to both EDTA 17% and Citric acid 40% across all *Staphylococcus*, *Streptococcus*, and

Candida strains ($p < 0.0001$). Besides, Maleic acid 6% exhibited significantly lower antimicrobial activity than Sodium hypochlorite 6% in several bacterial strains, including *S. aureus*, *S. epidermidis*, and *Streptococcus* spp. ($p = 0.0286$). These differences were consistent and robust, indicating a clear inferiority of Maleic acid in terms of antimicrobial performance.

In contrast, EDTA 17% with benzalkonium bromide (0.1%) and Citric acid 40% did not differ significantly from each other in any of the tested strains ($p = 0.999$), suggesting comparable antimicrobial efficacy. This equivalence was observed across both Gram-positive bacteria and fungal species, reinforcing the notion that both agents possess similar antimicrobial potential. The both agents demonstrated statistically significant superiority over Sodium hypochlorite 6% in antifungal activity, especially against *Candida albicans*, *C. tropicalis*, *C. lipolytica*, *C. lusitaniae*, and *C. kefyr* ($p = 0.0286$ for all). These findings highlight the potential of chelating agents and organic acids in targeting fungal pathogens, possibly due to their ability to disrupt biofilms or chelate essential ions.

These findings confirm that while EDTA and Citric acid are equally effective and broadly active, Maleic acid 6% is significantly less potent against a wide range of clinically relevant microorganisms. This has important implications for the selection of acidic irrigants in endodontic protocols, particularly when targeting polymicrobial infections.

The Kruskal–Wallis test followed by Dunn's post hoc analysis revealed that sodium hypochlorite 5.25% did not differ in antimicrobial activity from 6% NaOCl (both the standard formulation and Chemoplus) but was significantly more effective than 2% NaOCl ($p < 0.05$). Both chlorhexidine 2% solutions (Cerkamed and Endohex) demonstrated antimicrobial activity comparable to NaOCl formulations but markedly exceeded the efficacy of acidic and chelating agents (maleic acid, EDTA, and citric acid) for the majority of tested strains, particularly *Candida* spp. chlorhexidine ($p < 0.0001$). Welch's ANOVA with Tukey's HSD post hoc test confirmed these results, yielding similar conclusions regarding the equivalence of chlorhexidine and NaOCl and their superiority over acidic solutions. All *Candida* spp., as well as most *Staphylococcus* and *Streptococcus* strains, exhibited higher susceptibility to chlorhexidine-based formulations compared with acidic agents.

Figure 1 (Fig.1) summarizes the integral activity of irrigating solutions against bacterial and fungal strains from root canals used in the study. In general, NaOCl- and chlorhexidine-containing irrigation solutions showed comparable antibacterial activity. But sodium hypochlorite solutions 5.25% (Cerkamed) and 6%

(Chemoplus Chloran) both with and without detergent exceed significantly in their antifungal activity. Maleic acid and Citric acid showed almost no activity against root canal *Candida sp.* isolates.

NOVELTY OF THE RESEARCH

The study provides a direct, head-to-head comparison of an extended spectrum of endodontic irrigation solutions (10 formulations) antimicrobial activity under identical *in vitro* conditions against 13 clinical microbial strains associated with the apical periodontitis pathogenesis. The obtained results expand current understanding of the properties, potential advantages, and limitations of commonly used irrigants within the context of root canal infection control.

The findings provide a foundation for further investigations into potential synergistic interactions between individual irrigating agents and may contribute to the refinement of chemomechanical root canal treatment protocols, particularly in clinical situations involving mixed bacterial–fungal infections.

DISCUSSION

The present study demonstrated that sodium hypochlorite-based irrigation protocols exhibited the highest antimicrobial activity against microorganisms isolated from infected root canals of patients with apical periodontitis. The strongest effect was observed in the groups using 5.25% and 6% sodium hypochlorite, whereas the protocol based on 2% sodium hypochlorite showed lower efficacy against several tested strains. These findings are consistent with the results of Vianna et al. [6] and Gomes et al. [7], who reported pronounced antimicrobial activity of sodium hypochlorite against a broad spectrum of endodontic pathogens. Thus, our results confirm the well-established role of NaOCl as one of the most effective chemical irrigants in root canal disinfection. The superior antimicrobial performance of higher-concentration NaOCl observed in the present study can be explained by its stronger oxidative and proteolytic action, which promotes disruption of microbial cells and dissolution of organic tissue remnants. Similar observations have been reported in previous experimental studies, where higher concentrations of sodium hypochlorite showed increased antimicrobial potency, although the difference was not always proportional for all microbial groups [6,7]. At the same time, our findings are in agreement with contemporary reviews emphasizing that antimicrobial efficacy depends not only on concentration, but also on contact time, delivery technique, canal anatomy,

and irrigant activation [8,9]. An important result of the present study was the enhanced antifungal activity of the sodium hypochlorite formulation containing surfactants. This finding expands the existing evidence on modified hypochlorite formulations, as most previous studies have primarily focused on antibacterial rather than antifungal effects. The observed improvement may be explained by reduced surface tension and enhanced penetration of the irrigant into dentinal tubules and anatomical irregularities. This is particularly relevant given that *Candida spp.* have been repeatedly detected in infected and persistent root canal infections, as demonstrated by Rozas and Siqueira [10]. Therefore, our findings not only support previously reported antifungal efficacy of NaOCl [6], but also suggest that surfactant modification may provide an additional clinical advantage in fungal-associated endodontic infections. The antibacterial performance of chlorhexidine observed in the present study is also in line with previously published data. Basrani et al. [11] demonstrated the stability and antimicrobial substantivity of chlorhexidine, while Ballal et al. [12] and Prabhat et al. [13] confirmed its effectiveness against resistant endodontic microorganisms. In our study, CHX Endohex produced the largest inhibition zones against several bacterial strains, which is consistent with these reports. However, in agreement with the literature, chlorhexidine should not be regarded as a full substitute for sodium hypochlorite, because despite its strong antibacterial action, it lacks tissue-dissolving capacity and therefore cannot replace NaOCl as the principal irrigant [11–13].

The findings related to EDTA, citric acid, and maleic acid should also be interpreted in the context of previous studies. Our results showed that these agents demonstrated lower overall antimicrobial efficacy than sodium hypochlorite and chlorhexidine, which supports the concept that their primary role in endodontics is adjunctive rather than disinfectant. This interpretation is consistent with the conclusions of Tonini et al. [9] and Rath et al. [14], who emphasized that chelating and acidic solutions are mainly important for smear layer removal, dentin conditioning, and facilitation of irrigant penetration rather than for direct antimicrobial elimination. Therefore, the present study agrees with existing evidence that these agents should be incorporated into irrigation protocols as complementary components rather than primary antimicrobial solutions. The present findings are also consistent with more recent studies. Hackbarth et al. [15] confirmed the strong antimicrobial efficacy of sodium hypochlorite compared with newer irrigants, reinforcing its position as the gold standard in end-

odontic disinfection. Celikel et al. [16] also showed that NaOCl and chlorhexidine remain highly effective against *Enterococcus faecalis*, one of the most resistant microorganisms in endodontic infections. Furthermore, the umbrella review by Orozco-Gallego et al. [17] highlighted the clinical value of combining sodium hypochlorite with chelating agents, which supports the multimodal irrigation concept reflected in our findings. Taken together, the results of the present study both confirm and expand the available evidence regarding the antimicrobial performance of commonly used endodontic irrigants. Our data support previously reported superiority of sodium hypochlorite and chlorhexidine over acidic and chelating solutions, while also providing additional comparative information regarding surfactant-modified NaOCl formulations and their enhanced antifungal potential. This may be of particular clinical relevance in cases of persistent or mixed bacterial–fungal root canal infections. The main limitation of this study is its in vitro design and the absence of irrigant activation techniques, which may influence disinfecting performance under clinical conditions. Future research should focus on dynamic activation systems and advanced irrigant formulations, including nanoparticle-based and modified solutions, in order to improve

root canal disinfection and enhance the predictability of endodontic treatment outcomes.

CONCLUSIONS

The obtained microbiological findings allow for a differentiated selection of irrigation solutions during endodontic treatment. Chlorhexidine solutions demonstrated superior performance in clinical situations dominated by bacterial flora, whereas sodium hypochlorite remained essential for the effective elimination of fungal biofilms and mixed microbial infections. The comparatively limited antimicrobial potential of acidic and chelating agents confirms their auxiliary role rather than a primary disinfecting function, reinforcing the need for tailoring irrigant selection to the microbial characteristics of each case.

A rational irrigation strategy should integrate the complementary use of chemical agents to achieve broad-spectrum activity against polymicrobial biofilms commonly found in infected root canals. Future research should focus on identifying synergistic irrigant combinations and improving activation techniques to overcome the inherent limitations of in vitro models and enhance the predictability and long-term success of endodontic treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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