The Open Dentistry Journal
DOI: 10.2174/0118742106333707250515052904, 2025, 19, e18742106333707
ISSN: 1874-2106
1

RESEARCH ARTICLE
OPEN ACCESS

Comparative Evaluation of the Antimicrobial Efficacy of 20% Chlorhexidine, 3% Sodium Hypochlorite, and Dexamethasone Acetate with Thymol as a Root Canal Disinfectant against *Enterococcus faecalis*: An In-Vitro Feasibility Study

Trisha Gadekar[1], Raghavendra M. Shetty[2,3,4,*], Sunaina Shetty Yadadi[5], Pooja Adtani[6], Vijay B. Desai[1,2] and Kelvin I. Afrashtehfar[7,8,*]

[1]*Department of Pediatric Dentistry, Mamata Dental College, Khammam, TG, India*
[2]*Clinical Sciences Department, College of Dentistry, Ajman University, Ajman City P.O. Box 346, AJ, United Arab Emirates*
[3]*Center of Medical and Bio-allied Health Sciences Research, Ajman University, Ajman City P.O. Box 346, AJ, United Arab Emirates*
[4]*Department of Pediatric and Preventive Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Higher Education and Research (Declared as Deemed-to-be University), Wardha, MH, India*
[5]*Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, P.O. Box 27272Sharjah, SH, United Arab Emirates*
[6]*Department of Basic Medical and Dental Sciences, College of Dentistry, Gulf Medical University (GMU), P.O. Box 4184 Ajman City, AJ, United Arab Emirates*
[7]*Department of Reconstructive Dentistry and Gerodontology, School of Dental Medicine, University of Bern, 3010Berne, BE, Switzerland*
[8]*Private Practice Limited to Implant Dentistry, Richmond, BC, Canada*

Abstract:

Background: Endodontic treatment is aimed at eradicating bacteria from the root canal system and preventing their regrowth. Despite the widespread use of chlorhexidine gluconate, sodium hypochlorite, and steroids as root canal irrigants, the search for novel materials that enhance root canal disinfection remains ongoing.

Objective: This study aimed to evaluate the effectiveness of two newly introduced root canal irrigants, 20% chlorhexidine (Biosol) and dexamethasone acetate with thymol (Cresophene), compared to traditional irrigants, such as sodium hypochlorite.

Methods: Eighty uniradicular teeth extracted for periodontal and orthodontic reasons were divided into four groups: Group I (Biosol), Group II (Cresophene), Group III (3% NaOCl), and Group IV (Control). The teeth were inoculated with *Enterococcus faecalis*, treated with the respective irrigants, and sealed with sterile cotton pellets and temporary cement. After incubation at 37°C for 24 hours in Brain Heart Infusion (BHI) broth, microbiological sampling was performed at 48 hours to evaluate antimicrobial efficacy.

Results: Group I (Biosol) exhibited the highest antimicrobial efficacy with a significant reduction of 90.15% in *E. faecalis* counts, followed closely by Group II (Cresophene) with an 89.85% reduction. Group III (3% NaOCl) showed a comparatively lower reduction of 75.57%. Group IV (Control) demonstrated the highest bacterial presence, confirming its limited antimicrobial effectiveness. Statistical analysis revealed significant differences between Group I and Groups III and IV ($p < 0.0001$), with Group I being the most effective. Additionally, standard deviation analysis indicated variability in bacterial counts within each group.

Conclusion: This study suggests that 20% chlorhexidine (Biosol) and dexamethasone acetate with thymol (Cresophene) are highly effective as root canal irrigants. The remarkable substantivity of biosol helps establish a long-lasting antibacterial environment in the root canal, offering valuable potential for enhancing the success rates of endodontic treatments.

Keywords: Endodontic treatment, *Enterococcus faecalis*, Root canal irrigants, Root canal disinfection, Biosol, Bacterial eradication, Antimicrobial activity, Cresophene.

*Address correspondence to these authors at the Clinical Sciences Department, College of Dentistry, Ajman University, Ajman City P.O. Box 346, AJ, United Arab Emirates, Center of Medical and Bio-allied Health Sciences Research, Ajman University, Ajman City P.O. Box 346, AJ, United Arab Emirates, Department of Pediatric and Preventive Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Higher Education and Research (Declared as Deemed-to-be University), Wardha, MH, India, Department of Reconstructive Dentistry and Gerodontology, School of Dental Medicine, University of Bern, 3010 Berne, BE, Switzerland and Private Practice Limited to Implant Dentistry, Richmond, BC, Canada; E-mails: drraghavendra77@gmail.com, kelvin.afrashtehfar@unibe.ch and r.shetty@ajman.ac.ae

Cite as: Gadekar T, Shetty R, Yadadi S, Adtani P, Desai V, Afrashtehfar K. Comparative Evaluation of the Antimicrobial Efficacy of 20% Chlorhexidine, 3% Sodium Hypochlorite, and Dexamethasone Acetate with Thymol as a Root Canal Disinfectant against *Enterococcus faecalis*: An In-Vitro Feasibility Study. Open Dent J, 2025; 19: e18742106333707.
http://dx.doi.org/10.2174/0118742106333707250515052904

Received: September 18, 2024
Revised: April 15, 2025
Accepted: April 15, 2025
Published: May 16, 2025

1. INTRODUCTION

The continuous integration of novel technologies and materials fundamentally drives the relentless pursuit of improved patient outcomes in dentistry. From advanced imaging to biomimetic restorative materials, innovation is the cornerstone of progress across all dental disciplines, including pediatric and adult endodontics. The preservation of natural primary and permanent teeth through effective endodontic therapy remains paramount, especially when confronted with pulpal or periapical diseases [1, 2].

Over the past decades, pediatric and adult endodontics have witnessed significant advancements, particularly in irrigation and chemical disinfection. Effective irrigation and chemical agents have been instrumental in enhancing treatment success. However, despite these strides, the intricate and often inaccessible anatomy of the root canal system presents persistent challenges. Literature highlights that traditional mechanical instrumentation alone cannot achieve thorough disinfection, leaving behind reservoirs for bacteria, biofilms, and debris [3]. This realization has underscored the critical role of irrigation in reducing microbial load and removing tissue remnants.

Recent technological developments, such as advanced microscopy and imaging, have further revealed the limitations of current techniques, demonstrating that certain areas, especially in oval canals and isthmuses, remain untouched by instrumentation [4]. This necessitates the exploration and development of novel chemical irrigants to complement mechanical preparation, aiming for more complete disinfection.

The ideal intracanal medicament must exhibit a multifaceted profile, including potent antimicrobial activity, stability in biological fluids, sustained effects, low surface tension for optimal penetration, biocompatibility, and minimal risk of tooth discoloration or adverse immune responses [5]. While various irrigants exist, each with distinct mechanisms of action, challenges remain in finding a solution that balances efficacy with safety. Concerns regarding cell damage from apical extrusion [6] emphasize the need for rigorous evaluation of new irrigants.

Chlorhexidine gluconate, a widely used irrigant, has demonstrated broad-spectrum antimicrobial activity [7]. However, the specific application of 20% chlorhexidine requires further investigation. Similarly, the use of steroids, such as dexamethasone acetate, in endodontics for inflammation and pain management is well-established [8], but their combination with agents like thymol (cresophene) as an irrigant is less explored.

The present study builds upon the existing literature by investigating the efficacy of two novel irrigants, 20% chlorhexidine (biosol) and dexamethasone acetate with thymol (cresophene), and comparing them to the gold standard, 3% sodium hypochlorite. Specifically, this study aims to address the knowledge gap regarding the antimicrobial effectiveness of these novel irrigants, offering a comparative analysis that goes beyond conventional approaches. The primary objective is to evaluate their efficacy in a controlled experimental setting, thereby contributing to developing evidence-based protocols for endodontic therapy. The null hypothesis postulates that there is no significant difference in the antimicrobial activity of these irrigants. By testing this hypothesis, we aim to identify the most effective irrigant, ultimately enhancing treatment outcomes and patient care. The significance of this study lies in its potential to expand the current understanding of root canal disinfection and provide clinicians with valuable insights for improved endodontic practice in pediatric and adult patients.

2. MATERIALS AND METHODS

This study aimed to compare the effectiveness of three different disinfection methods in reducing *Enterococcus faecalis* (*E. faecalis)*, a common endodontic pathogen, in extracted human teeth. The study was approved by the ethical committee of the institute where it was conducted (Ref. no. CDCRI. 28/18). A total of 80 single-rooted permanent teeth, extracted for orthodontic and periodontal reasons, were selected for the study. The teeth were cleaned using a 5.2% NaOCl solution for 30 minutes to remove organic matter and then stored in a physiologic saline solution until the process began. The crowns were sectioned at the cementoenamel junction using a high-density diamond disc to obtain a uniform root canal length of 15mm. Access openings were made using endo access burs, and pre-coronal flaring was performed with Gates Glidden drills. The root canals were then prepared using the step-back technique (up to the #35 K file; 25 mm, Dentsply-Maillefer). After each instrumentation, the canals were irrigated using physiological saline solution and 5 mL of 3% sodium hypochlorite solution. EDTA gel was applied for 2-3 minutes to remove the smear layer.

2.1. Preparation of Inoculum

The bacterial strain *E. faecalis ATCC 29212* from HiMedia Labs, Pune, was used in this study. The cell density was adjusted to 9.7 x 10^8 cells/mL for each test group. Microbiological sampling was carried out before the application of medicaments. The test microorganisms were maintained on Brain Heart Infusion (BHI) agar slants at 40°C. An overnight BHI broth liquid culture of the organisms was prepared for each experiment. Microbiological sampling was conducted under sterile conditions to ensure that the teeth were free of bacterial contamination before the experiment. The teeth were autoclaved at 121°C for 20 minutes. The media was diffused in refined water and autoclaved at 15 lbs. pressure (121°C) for 15 minutes. The sterile teeth roots were inoculated with 10 µL of inoculum using a micropipette and then sealed with temporary cement after the placement of sterile cotton soaked with inoculum in the cervical access of the canal. The teeth were then incubated overnight at 37°C for 24 hours. The CFU of *E. faecalis ATCC 29212* was 500,000.

2.2. Procedure of Disinfection

The root canals of 80 teeth were dried with paper points and randomly divided into four groups, with 20 teeth in each group (Fig. **1**). Group I received disinfection with 20% chlorhexidine (biosol). Group II received disinfection with dexamethasone acetate plus thymol (Cresophene). Group III received disinfection with 3% sodium hypochlorite, while Group IV served as the control group without any disinfection. Each group received 0.2 mL of the respective medicament, which was injected into the canals using a 26-gauge needle. To differentiate between the groups, a layer of colored enamel was applied to the teeth, and the apical foramen was sealed from the outside using nail varnish.

2.3. Assessment of CFU

After incubation for 48 hours, the root canals were cleaned and irrigated with saline, and then instrumentation was performed using a #45 file (25mm, Dentsply-Maillefer) to obtain dentinal shavings. The canals were again filled with physiological saline. Sterile paper points were placed in the canals for 30 seconds to collect the samples. The paper points were then placed into 1 mL of saline in test tubes, which were vigorously shaken and diluted to determine the colony-forming units per milliliter (CFU/mL). The survival of *E. faecalis* in the root canals of four experimental groups was quantified by determining colony-forming units (CFU). Aliquots from each sample were processed using the pour-plate method to estimate CFU/mL, and optical density (OD) at 600 nm was also measured. Survival fractions in each root specimen were calculated by counting the colonies on the experimental plates and dividing by the number of colonies from controls. Overall, this study aimed to evaluate the effectiveness of three different disinfection methods in reducing *E. faecalis* in extracted human teeth. The experimental design involved sterilizing, inoculating, and incubating the teeth with the test microorganisms, followed by applying the disinfection agents and incubating the specimens. The survival fractions of *E. faecalis* were then measured using colony-forming units and optical density (Fig. **2**). The findings of this study could help inform clinicians when selecting disinfection agents for root canal treatment.

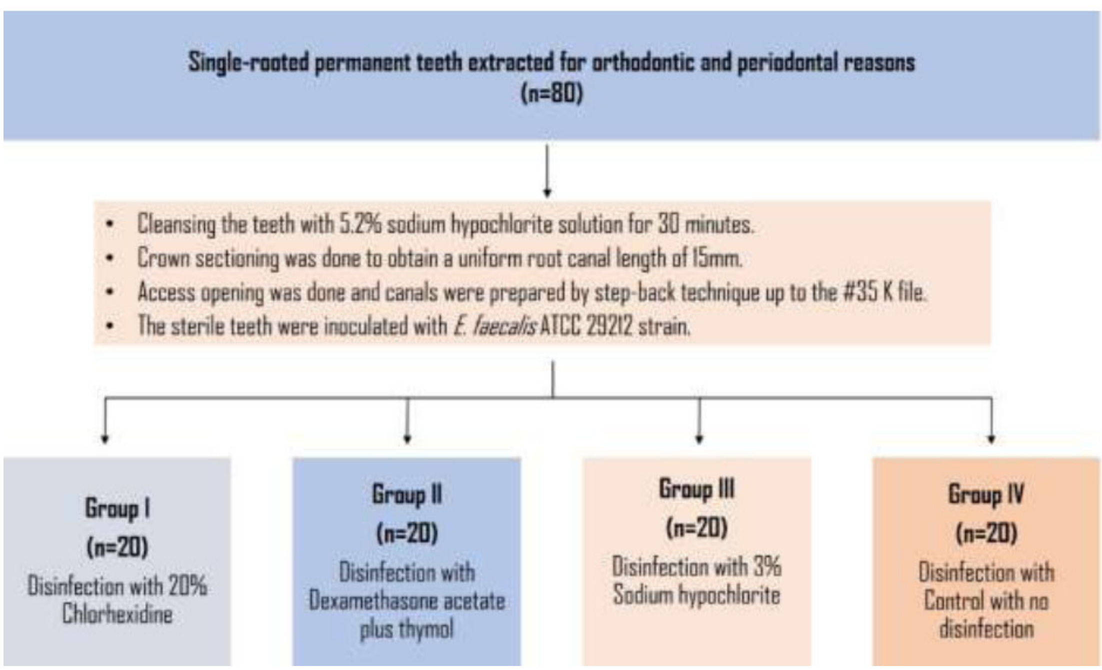

Fig. (1). Flowchart illustrating the steps used in the material and methods.

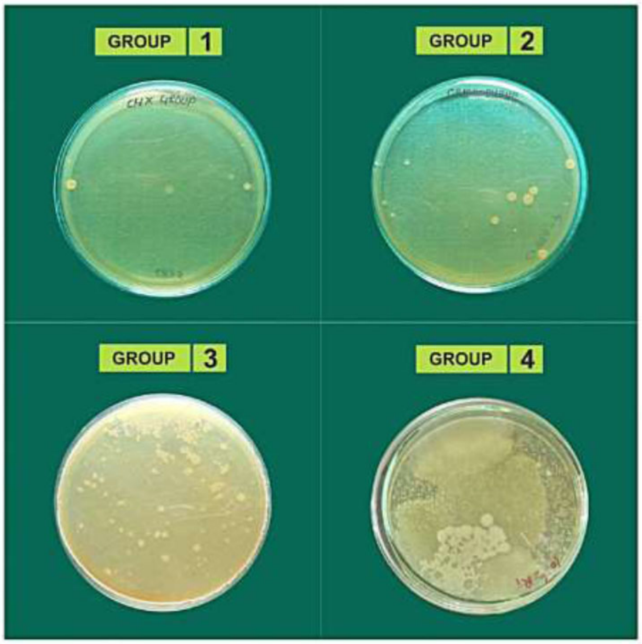

Fig. (2). Colonies formed by *E. faecalis*.

2.4. Statistical Analysis

The data on counts of endodontic pathogens, *i.e.*, *E. faecalis,* was obtained for 20 samples from each of the study groups. The mean and median values of the endodontic pathogen, *i.e.*, *E. faecalis*, were obtained for all the study samples. The comparison of data across the groups was performed using a one-way analysis of variance (ANOVA) test. The pairwise comparison of counts between groups was carried out using Tukey's post hoc test. The statistical significance was tested at 5%, and the analysis was performed using SPSS 20.0 ver. (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Distribution of E. faecalis among Study Groups

Table **1** presents the distribution of *E. faecalis* counts across the study groups, offering a visual representation of our investigation. Each group's *E. faecalis* count was measured, and the relative standard deviation was calculated to assess variability. Group I had the smallest *E. faecalis* count, with a mean of 7.94 CFU/ml and a relative standard deviation of 2.26, indicating lower bacterial presence on average than the other groups. Group II had a mean count of 7.97 CFU/ml and a relative standard deviation of 1.37, suggesting relatively less variability than Group I. Group III recorded a mean count of 8.37 CFU/ml and a relative standard deviation of 0.51, showing the least variability among the groups. Group IV had the highest mean count of *E. faecalis*, with 9.28 CFU/ml and a relative standard deviation of 0.91. Significant differences in *E. faecalis* counts were observed among all groups ($p < 0.0001$), indicating experimental manipulation as the likely cause. The lowest mean count of *E. faecalis* was found in Group I, with significant differences noted among all groups. Relative standard deviation values provided additional insight into variability within each group.

Table 1. Distribution of *E. faecalis* counts across the study groups.

Groups	*E. faecalis* Count	Significance	
	Mean ± SD	*F - value*	*p*-value
Group I	7.94 ± 2.26	587.3	< 0.0001
Group II	7.97 ± 1.37		
Group III	8.37 ± 0.51		
Group IV	9.28 ± 0.91		

3.2. Comparison of E. faecalis within Study Groups

Table **2** presents the results of a study comparing the efficacy of various disinfectants in reducing *E. faecalis* counts. Mean counts and standard deviations were calculated for each group to assess differences. Group I had a mean count of 7.94 ± 2.26, and Group II had 7.97 ± 1.37, with a non-significant *p*-value of 0.72, indicating no difference. Group III (8.37 ± 0.51) had significantly fewer counts than Group II ($p < 0.0001$), suggesting the superiority of biosol over NaOCl. Group IV (9.28 ± 0.91) had significantly more counts than Group I ($p < 0.0001$), implying that the disinfectant of Group IV was less effective. Group II (7.97 ± 1.37) had significantly fewer counts than Group IV ($p < 0.0001$), indicating the superiority of cresophene over NaOCl. Biosol was more effective than NaOCl, and Group IV had the highest *E. faecalis* count. Standard deviation values offered insights into result variability within groups.

Table 2. Comparison of *E. faecalis* (CFU's/ml) within the study groups.

Groups			*E. faecalis*	
			Mean ± SD	*p*-value
Group I	*vs.*	Group II	7.97 ± 1.37	0.7228
		Group III	8.37± 0.51	< 0.0001
		Group IV	9.28 ± 0.91	< 0.0001
Group II	*vs.*	Group III	8.37 ± 0.51	< 0.0001
		Group IV	9.28 ± 0.91	< 0.0001
Group III	*vs.*	Group IV	9.28 ± 0.91	< 0.0001

3.3. Comparing Disinfectant Efficacy against E. faecalis

Table **3** presents the outcomes of a study evaluating the efficacy of diverse disinfectants in decreasing *E. faecalis* counts in root canals. The log reductions in the bacterial count were calculated as Log Reduction=log10 (A/ B), where 'A' denotes the number of viable microorganisms before treatment and 'B' denotes the number of viable microorganisms after treatment. The investigation comprised four groups, each exhibiting a distinct mean count of *E. faecalis*. Group IV notably displayed a significantly higher count compared to Group III. Additionally, the study reported mean reductions in *E. faecalis* count for each group; Group I achieved a mean reduction of 90.15 ± 4.78%, Group II achieved 89.85 ± 2.77%, and Group III achieved 75.57 ± 2.46%. Notably, 20% CHX demonstrated the most substantial reduction, while 3% sodium hypochlorite exhibited the least reduction. These findings underscore the significant influence of disinfectant type and concentration on reducing *E. faecalis* count in root canals, with cresophene proving more effective than NaOCl and 20% CHX demonstrating the highest reduction.

Table 3. Reduction in *E. faecalis* count.

Groups	Reduction in *E. faecalis* Count (%)
Group I	90.15%
Group II	89.85%
Group III	75.57%

4. DISCUSSION

The use of antimicrobial medicaments in endodontic treatment is pivotal for the success of root canal therapy by eliminating microorganisms from the root canal space [9]. However, persistent periapical lesions caused by microorganisms like *E. faecalis* pose challenges due to their biofilm formation, which shields them against traditional treatment methods and resistance to antimicrobial agents [10, 11]. Additionally, the emergence of multidrug-resistant *E. faecalis* strains underscores the need for effective intraradicular disinfectants [12]. In this study, we evaluated the efficacy of different root canal disinfectants using the *E. faecalis* 29212 strains. One disinfectant, chlorhexidine, well-known for its broad-spectrum antibacterial properties, exhibited promising results due to its immediate antimicrobial action, biocompatibility, and mode of action involving Sortase A protein interference [13-17]. We used a higher concentration, *i.e.*, 20% chlorhexidine (biosol), known for enhanced bactericidal activity and stability at a broader pH range [18, 19]. Chlorhexidine at concentrations above 2%, combined with Cetrimide (CTR), can eliminate *E. faecalis* similar to 2.5% NaOCl [20]. However, lower concentrations (0.2%) may exhibit a bacteriostatic effect by inhibiting membrane function [21, 22]. Despite limited literature on 20% chlorhexidine, we chose it for its potential efficacy. The long-lasting substantivity of chlorhexidine refers to its ability to bind to dentine and exert a prolonged antimicrobial effect, making it valuable in endodontics, where disinfection of the root canal system is crucial [13, 14, 23]. One of the main arguments favoring CHX is its ability to bind to dentine and exert a prolonged antimicrobial effect (substantivity), which may prevent bacterial recolonization after root canal treatment. In cases of severe root canal infection, particularly those exhibiting sinus tracts, purulence, or requiring retreatment, final irrigation with 2% chlorhexidine is recommended [24].

Moreover, the present study explored cresophene, a combination of dexamethasone acetate and thymol, which achieved an 89.95% reduction in *E. faecalis* counts, comparable to biosol, suggesting its effectiveness in root canal disinfection. Corticosteroids in cresophene contribute to inflammation reduction and membrane stabilization, reducing post-operative pain [24]. Thymol, an antifungal agent, enhances disinfection by altering membrane permeability and has demonstrated antimicrobial activity against *E. faecalis* [25-27]. Camphor oil, containing cinnamaldehyde and other antimicrobial compounds, shows promise as an alternative root canal disinfectant [28, 29]. Still, further research is required to evaluate its effectiveness. Sodium hypochlorite (NaOCl), a common root canal irrigant with broad-spectrum antimicrobial activity, is effective but carries the risk of adverse effects, including tissue damage, pain, and tissue discoloration [30-39]. In our study, lower concentrations of sodium hypochlorite (3% NaOCl) resulted in a 75% reduction in *Enterococcus faecalis* counts. However, this concentration has limitations and may lead to potential adverse effects. As a result, alternative disinfectants, such as 20% chlorhexidine and cresophene, may provide additional benefits in root canal therapy, meeting the demand for effective and safe intraradicular disinfectants [18, 19].

4.1. Study Limitations and Scope for Further Research

While the present *in-vitro* study demonstrates the potential efficacy of novel irrigants (20% chlorhexidine and dexamethasone acetate with thymol) against E. faecalis, it presents several limitations that necessitate cautious interpretation and guide future research directions. Firstly, the inherent constraints of an *in vitro* model limit its ability to fully replicate the complex and dynamic environment of the clinical setting. Consequently, direct extrapolation of these findings to *in-vivo* conditions should be approached with prudence.

Secondly, the study's focus on *E. faecalis* as the sole target microorganism, while clinically relevant, does not reflect the polymicrobial nature of endodontic infections. Future investigations should expand the microbiological spectrum to include a wider range of bacterial species commonly encountered in root canal systems, thus providing a more comprehensive assessment of disinfectant efficacy.

Thirdly, the use of 20% chlorhexidine, a concentration exceeding typical clinical applications, raises concerns regarding its safety profile. Potential adverse effects, such as tissue damage and allergic reactions, warrant further evaluation. Future studies should explore varying concentrations of chlorhexidine and investigate alternative intracanal medicaments to optimize both efficacy and safety.

Furthermore, the absence of cytotoxicity assessments in this study represents a critical limitation. Given the potential for these disinfectants to interact with periapical tissues, future research must incorporate comprehensive cytotoxicity evaluations to ensure their biocompatibility and safety for clinical use.

To advance our understanding of root canal disinfection and translate these *in-vitro* findings into clinically relevant protocols, we recommend the following:

- Expanding the microbiological evaluation to include a broader spectrum of microorganisms relevant to endodontic infections.
- Performing comprehensive cytotoxicity assessments to determine the biocompatibility of these disinfectants.
- Conducting well-designed clinical trials to validate the efficacy and safety of these disinfectants *in vivo*.

Future research addressing these limitations and recommendations is essential for enhancing our understanding of root canal disinfection and ultimately improving treatment outcomes in endodontic practice.

CONCLUSION

Within the limitations of this *in-vitro* feasibility study, 20% chlorhexidine (biosol) and dexamethasone acetate with thymol (cresophene) demonstrated effective disinfection of root canals. Notably, the substantivity of 20% chlorhexidine suggests its potential for prolonged antibacterial effects. Nevertheless, these findings require validation through *in-vivo* investigations. Future research should incorporate comprehensive cytotoxicity evaluations to ensure their biocompatibility and safety for clinical use.

AUTHORS' CONTRIBUTIONS

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethical committee of the institute where it was conducted (Ref. no. CDCRI. 28/18).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained for this study.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Government V.Y.T.P.G. Autonomous College (College of Arts and Sciences) and Dr. Dhananjay Raje, Head, Data Analytics Division at MDS Bio-Analytics Pvt. Ltd. Nagpur.

REFERENCES

[1] Murvindrann V, Raj J. Antibiotics as an intracanal medicament in endodontics. Pharm Sci and Res 2013; 6(9): 297-301.

[2] Dioguardi M, Gioia GD, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent 2018; 12(3): 459-66. http://dx.doi.org/10.4103/ejd.ejd_56_18 PMID: 30147418

[3] Elmsmari F, Prina JN, Morales MNP, *et al.* Post-instrumentation dentinal microcracks induced by Two NiTi rotary systems with increased super elasticity and shape memory: A MicroCT comparative and methodological *ex vivo* study. Cosmetics 2023; 10(1): 37. http://dx.doi.org/10.3390/cosmetics10010037

[4] Boutsioukis C, Psimma Z, van der Sluis LWM. Factors affecting irrigant extrusion during root canal irrigation: a systematic review. Int Endod J 2013; 46(7): 599-618. http://dx.doi.org/10.1111/iej.12038 PMID: 23289914

[5] Kar P, Varghese R, Agrawal N, Jhaveri H. Steroid as an Intracanal Medicament: An Advanced Review. Journal of Research in Dental and Maxillofacial Sciences 2021; 6(3): 47-51. http://dx.doi.org/10.52547/jrdms.6.3.47

[6] Gomes BPFA, Vianna ME, Zaia AA, Almeida JFA, Souza-Filho FJ, Ferraz CCR. Chlorhexidine in Endodontics. Braz Dent J 2013; 24(2): 89-102. http://dx.doi.org/10.1590/0103-6440201302188 PMID: 23780357

[7] Del Fabbro M, Afrashtehfar KI, Corbella S, El-Kabbaney A, Perondi I, Taschieri S. *In vivo* and *in vitro* effectiveness of ro-tary nickel-titanium *vs* manual stainless steel instruments for root canal therapy: Systematic review and meta-analysis. J Evid Based Dent Pract 2018; 18(1): 59-69. http://dx.doi.org/10.1016/j.jebdp.2017.08.001 PMID: 29478682

[8] El karim I, Kennedy J, Hussey D. The antimicrobial effects of root canal irrigation and medication. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103(4): 560-9. http://dx.doi.org/10.1016/j.tripleo.2006.10.004 PMID: 17223590

[9] Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008; 34(11): 1291-1301.e3. http://dx.doi.org/10.1016/j.joen.2008.07.028 PMID: 18928835

[10] Teixeira LM, Facklam RR. "Enterococcus,"in Topley and Wilson's Microbiology and Microbial Infections. Chichester, UK: John Wiley & Sons 2005.

[11] Rôças I, Siqueira J Jr, Santos K. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30(5): 315-20. http://dx.doi.org/10.1097/00004770-200405000-00004 PMID: 15107642

[12] Baldassarri L, Creti R, Montanaro L, Orefici G, Arciola CR. Pathogenesis of implant infections by enterococci. Int J Artif Organs 2005; 28(11): 1101-9. http://dx.doi.org/10.1177/039139880502801107 PMID: 16353116

[13] Greenstein G, Jaffin RA, Hilsen KL, Berman CL. Repair of anterior gingival deformity with durapatite. A case report. J Periodontol 1985; 56(4): 200-3. http://dx.doi.org/10.1902/jop.1985.56.4.200 PMID: 2987474

[14] aEmilson CG. Susceptibility of various microorganisms to chlorhexidine. Scand J Dent Res 1977; 85(4): 255-65. http://dx.doi.org/10.1111/j.1600-0722.1977.tb00561.x PMID: 266752 ; bArias CA. Management of multidrug-resistant enterococcal infections. Clin Microbiol Infect 2010 Jun; 16(6): 555-62. http://dx.doi.org/10.1111/j.1469-0691.2010.03214.x

[15] Yan Y, Zhou P, Lu H, *et al.* Potential apply of hydrogel-carried chlorhexidine and metronidazole in root canal disinfection. Dent Mater J 2021; 40(4): 986-93. http://dx.doi.org/10.4012/dmj.2020-299 PMID: 33883328

[16] Daood U, Matinlinna JP, Pichika MR, Mak KK, Nagendrababu V, Fawzy AS. A quaternary ammonium silane antimicrobial triggers bacterial membrane and biofilm destruction. Sci Rep 2020; 10(1): 10970. http://dx.doi.org/10.1038/s41598-020-67616-z PMID: 32620785

[17] Makvandi P, Jamaledin R, Jabbari M, Nikfarjam N, Borzacchiello A. Antibacterial quaternary ammonium compounds in dental materials: A systematic review. Dent Mater 2018; 34(6): 851-67. http://dx.doi.org/10.1016/j.dental.2018.03.014 PMID: 29678327

[18] Hennessey TD. Some antibacterial properties of chlorhexidine. J Periodontal Res 1973; 8(s12): 61-7. http://dx.doi.org/10.1111/j.1600-0765.1973.tb02166.x

[19] Rölla G, Melsen B. On the mechanism of the plaque inhibition by chlorhexidine. J Dent Res 1975; 54(2_suppl): 57-62. http://dx.doi.org/10.1177/00220345750540022601 PMID: 237021

[20] Tanomaru-Filho M, Nascimento CA, Faria-Junior NB, Faria G, Guerreiro-Tanomaru JM. Antimicrobial activity of root canal irrigants associated with cetrimide against biofilm and planktonic Enterococcus faecalis. J Contemp Dent Pract 2014; 15(5): 603-7. http://dx.doi.org/10.5005/jp-journals-10024-1586 PMID: 25707833

[21] Greenstein G, Berman C, Jaffin R. Chlorhexidine. An adjunct to periodontal therapy. J Periodontol 1986; 57(6): 370-7. http://dx.doi.org/10.1902/jop.1986.57.6.370 PMID: 3522851

[22] Dupper A, Chandrappa PM, Tripathi P, Arroju R, Sharma P, Sulochana K. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against Enterococcus faecalis in Endodontics: An *in vitro* study. J Int Soc Prev Community Dent 2015; 5(8) (Suppl. 2): 89. http://dx.doi.org/10.4103/2231-0762.172952 PMID: 26942123

[23] Boutsioukis C, Arias-Moliz MT. Present status and future directions – Irrigants and irrigation methods. Int Endod J 2022; 55(S3) (Suppl. 3): 588-612. http://dx.doi.org/10.1111/iej.13739 PMID: 35338652

[24] Zou X, Zheng X, Liang Y, *et al*. Expert consensus on irrigation and intracanal medication in root canal therapy. Int J Oral Sci 2024; 16(1): 23. http://dx.doi.org/10.1038/s41368-024-00280-5 PMID: 38429299

[25] Seltzer S, Naidorf I. Flare-ups in endodontics: II. Therapeutic measures. 1985. J Endod 2004; 30(7): 482-8. http://dx.doi.org/10.1097/00004770-200407000-00006 PMID: 15220642

[26] Nostro A, Blanco AR, Cannatelli MA, *et al*. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol Lett 2004; 230(2): 191-5. http://dx.doi.org/10.1016/S0378-1097(03)00890-5 PMID: 14757239

[27] Ali IAA, Neelakantan P. Antibiofilm activity of phytochemicals against *Enterococcus faecalis* : A literature review. Phytother Res 2022; 36(7): 2824-38. http://dx.doi.org/10.1002/ptr.7488 PMID: 35522168

[28] Martínez-Pabón MC, Ortega-Cuadros M. Thymol, menthol and eucalyptol as agents for microbiological control in the oral cavity: A scoping review. Rev Colomb Cienc Quím Farm 2020; 49(1): 44-69.

[29] Chang ST, Chen PF, Chang SC. Antibacterial activity of leaf essential oils and their constituents from Cinnamomum osmophloeum. J Ethnopharmacol 2001; 77(1): 123-7. http://dx.doi.org/10.1016/S0378-8741(01)00273-2 PMID: 11483389

[30] Lincoln DE, Lawrence BM. The volatile constituents of camphorweed, heterotheca subaxillaris. Phytochemistry 1984; 23(4): 933-4. http://dx.doi.org/10.1016/S0031-9422(00)85073-6

[31] Tilakchand M, Hegde S, Naik B. Evaluation of the efficacy of a novel antibiotic–steroid paste *versus* conventionally used intracanal antibiotic pastes and irrigating solutions against a 3-week-old biofilm of *Enterococcus faecalis.* J Conserv Dent 2020; 23(5): 436-40. http://dx.doi.org/10.4103/JCD.JCD_304_19 PMID: 33911349

[32] Radcliffe CE, Potouridou L, Qureshi R, *et al*. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. Int Endod J 2004; 37(7): 438-46. http://dx.doi.org/10.1111/j.1365-2591.2004.00752.x PMID: 15189432

[33] Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J 2002; 13(2): 113-7. http://dx.doi.org/10.1590/S0103-64402002000200007 PMID: 12238801

[34] Behrents KT, Speer ML, Noujeim M. Sodium hypochlorite accident with evaluation by cone beam computed tomography. Int Endod J 2012; 45(5): 492-8. http://dx.doi.org/10.1111/j.1365-2591.2011.02009.x PMID: 22283726

[35] Witton R, Henthorn K, Ethunandan M, Harmer S, Brennan PA. Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. Int Endod J 2005; 38(11): 843-8. http://dx.doi.org/10.1111/j.1365-2591.2005.01017.x PMID: 16218978

[36] Pelka M, Petschelt A. Permanent mimic musculature and nerve damage caused by sodium hypochlorite: A case report. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 106(3): e80-3. http://dx.doi.org/10.1016/j.tripleo.2008.05.003 PMID: 18602848

[37] Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in Endodontics. Dent Clin North Am 2010; 54(2): 291-312. http://dx.doi.org/10.1016/j.cden.2009.12.001 PMID: 20433979

[38] Bahena CA. Use of sodium hypochlorite in root canal irrigation. Rev Odontol Mex 2012; 16(4): 252-8.

[39] Jaiswal N, Sinha DJ, Singh UP, Singh K, Jandial UA, Goel S. Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on *Enterococcus faecalis* biofilm : An *in vitro* study. J Clin Exp Dent 2017; 9(9): 0. http://dx.doi.org/10.4317/jced.53777 PMID: 29075407