Original Research

Comparative Evaluation of Antimicrobial Efficacy of Contemporary and Novel Intracanal Medicaments against Enterococcus Faecalis - An *In-vitro* Study

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Abstract

Background: Endodontic treatment aims to preserve compromised teeth by eliminating microorganisms from the root canal system. Persistent root canal infections, especially those involving Enterococcus faecalis (E. faecalis), can lead to treatment failure. This study evaluates the antimicrobial efficacy of both traditional and novel intracanal medicaments against E. faecalis. **Materials and Methods:** The study employed an in-vitro design to assess the antimicrobial efficacy of various intracanal medicament combinations against E. faecalis (ATCC 29212). The medicaments tested included 2% Chlorhexidine (CHX) combined with Alexidine, Calcium Hydroxide ($Ca(OH)_2$), Metronidazole, and Silver Nanoparticles (AgNPs). Antimicrobial activity was determined using the disc diffusion method, and the zone of inhibition (ZOI) was measured at 24, 48, and 72 hours. **Results:** Group 1 (2% CHX + Alexidine) and Group 5 (2% CHX + $Ca(OH)_2$) showed the highest initial antimicrobial efficacy at 24 hours, with mean ZOIs of 18.16 mm and 18.09 mm, respectively. At 48 and 72 hours, Group 4 (2% CHX + AgNPs) exhibited the highest efficacy with a mean ZOI of 16.17 mm. Statistical analysis showed significant differences between groups at all time intervals ($P < 0.05$). **Conclusion:** The combination of traditional and novel intracanal medicaments, specifically CHX with Alexidine and CHX with AgNPs, significantly enhanced antimicrobial efficacy against E. faecalis. The combination of CHX and Alexidine demonstrated the highest initial activity, while CHX and AgNPs provided sustained antimicrobial effects.

Keywords: *Alexidine, antimicrobial efficacy, chlorhexidine, endodontic treatment, Enterococcus faecalis, intracanal medicaments, root canal therapy, silver nanoparticles*

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Introduction

Endodontic treatment aims to preserve compromised teeth by eliminating bacteria from the root canal system, thus creating an environment favorable for periodontal healing.[1,2] Ideal root canal therapy involves creating straight-line access, performing chemomechanical preparation, and achieving three-dimensional obturation to form a hermetic seal that prevents reinfection and ensures long-term prognosis.[3] However, due to complex root canal anatomy and polymicrobial infections, standard instrumentation and irrigants may sometimes be insufficient to achieve a bacteria-free canal space.[4]

Root canal infections are often polymicrobial, with *Enterococcus faecalis* (*E. faecalis*) frequently associated with persistent and recurrent infections. *E. faecalis*, a Gram-positive facultative anaerobe, can resist many root canal irrigants due to its ability to form biofilms on dentin. While intracanal medicaments such as calcium

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hydroxide, chlorhexidine, and antibiotics are used to eradicate these persistent microorganisms, no single medicament is entirely effective against the diverse root canal microbiota. This study evaluates the antimicrobial efficacy of traditional intracanal medicaments, such as chlorhexidine, calcium hydroxide, and metronidazole, along with newer agents like Alexidine and silver nanoparticles, against *E. faecalis*.

Materials and Methods

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Saraswati Dental College and Hospital, Lucknow, India. The experimental work was carried out in the Department of Microbiology at King George's Medical University, Lucknow. The study received ethical clearance from the Institutional Research and Developmental Committee, with approval number #SD2CE21022022D.

The present in-vitro study evaluated the antimicrobial efficacy of contemporary and novel intracanal medicaments against Enterococcus faecalis. The materials used included Muller Hinton agar media and E. faecalis (ATCC 29212). Six groups were tested: Group 1 (2% CHX + Alexidine), Group 2 (Calcium Hydroxide + Alexidine), Group 3 (2% CHX + Metronidazole), Group 4 (2% CHX + Silver Nanoparticles), Group 5 (2% CHX + Calcium Hydroxide), and Group 6 (Saline control).

The antimicrobial activity was assessed using the disc diffusion method. Each medicament was impregnated onto paper disks and placed on inoculated agar plates, which were incubated at 37°C for 24, 48, and 72 hours. Zones of inhibition were measured to determine efficacy. This methodology enabled the comparative evaluation of these medicaments individually and in combination against E. faecalis in an in-vitro setting [Figure 1].

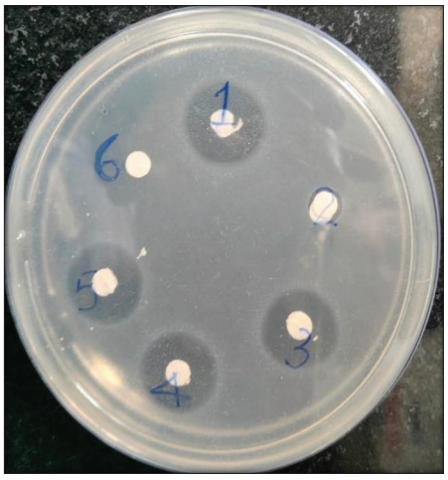

Figure 1: Zone of inhibition of intracanal medicaments against E.faecalis at 72 hours

Results

The antimicrobial efficacy of various intracanal medicament combinations against *Enterococcus faecalis* was evaluated by measuring the zone of inhibition (ZOI)

Table 1: Zone of inhibition (ZOI) measured at different time intervals

Group	Time Intervals	Mean ZOI (mm)±SD
Group 1 (2% CHX + Alexidine)	24 hours	18.16±0.05
	48 hours	14.11±0.09
	72 hours	14.11±0.09
Group 2 (Alexidine + Ca $(OH)_2$)	24 hours	3.06±0.04
	48 hours	2.11±0.12
	72 hours	2.05±0.10
Group 3 (2% CHX + Metronidazole)	24 hours	15.31±0.21
	48 hours	11.16±0.21
	72 hours	10.49±0.24
Group 4 (2% CHX + AgNPs)	24 hours	16.22±0.08
	48 hours	16.17±0.09
	72 hours	16.17±0.09
Group 5 (2% CHX + $Ca(OH)_2$)	24 hours	18.09±0.09
	48 hours	16.13±0.07
	72 hours	16.00±0.03
Group 6 (Saline - Control)	24 hours	0.00±0.00
	48 hours	0.00±0.00
	72 hours	0.00±0.00

Table 2: Comparative evaluation of antimicrobial efficacy between groups at 24 hours (one-way ANOVA)

Group Comparisons	Mean Difference	Significance (*P*)
Group 1 vs Group 2	15.10	0.000*
Group 1 vs Group 3	2.85	0.000*
Group 1 vs Group 4	1.94	0.000*
Group 1 vs Group 5	0.07	0.493 (NS)
Group 2 vs Group 3	-12.25	0.000*
Group 2 vs Group 4	-13.16	0.000*
Group 2 vs Group 5	-15.02	0.000*
Group 3 vs Group 4	-0.91	0.000*
Group 4 vs Group 5	-1.87	0.000*

*Significant; NS=Not Significant

Table 3: Comparative evaluation of antimicrobial efficacy at 72 hours (one-way ANOVA)

Group Comparisons	Mean Difference	Significance (*P*)
Group 1 vs Group 2	12.06	0.000*
Group 1 vs Group 3	3.62	0.000*
Group 1 vs Group 4	-2.06	0.000*
Group 1 vs Group 5	-1.89	0.000*
Group 2 vs Group 3	-8.44	0.000*
Group 2 vs Group 4	-14.12	0.000*
Group 2 vs Group 5	-13.95	0.000*
Group 3 vs Group 4	-5.68	0.000*
Group 4 vs Group 5	0.17	0.005*

*Significant; NS=Not Significant

in millimeters after 24, 48, and 72 hours of incubation [Table 1]. The study used a total of 13 samples per group to ensure the reliability of the results, and the data was statistically analyzed using one-way ANOVA and Tukey's *post hoc* test.

- At 24 hours, Group 1 (2% CHX + Alexidine) and Group 5 (2% CHX + Calcium Hydroxide) showed the highest antimicrobial efficacy, with a mean zone of inhibition of 18.16 mm and 18.09 mm, respectively.
- At 48 and 72 hours, Group 4 (2% CHX + Silver Nanoparticles) showed the highest antimicrobial efficacy, with a mean zone of inhibition of 16.17 mm.
- Group 6 (Saline) showed no antimicrobial activity at any time interval, confirming its role as the negative control.
- Statistical analysis using one-way ANOVA and Tukey's *post hoc* test showed significant differences between the groups at all time intervals, indicating that the combination of medicaments significantly impacts their antimicrobial efficacy against *E. faecalis* [Tables 2 and 3].

Discussion

The present study highlights the potential benefits of combining chlorhexidine (CHX) with other antimicrobial agents to enhance the efficacy of root canal therapy. The combination of CHX with alexidine (ALX) exhibited the highest initial antimicrobial activity against E. faecalis, with a mean zone of inhibition of 18.16 mm at 24 hours. This effectiveness is likely due to the synergistic properties of CHX and ALX, both bis-biguanides with potent disinfectant capabilities. CHX disrupts the bacterial cell wall, facilitating the penetration of ALX into the cytoplasm, leading to intracellular destruction.[3] This synergistic effect could be highly beneficial in routine root canal therapy, particularly in cases where conventional treatments fail to eradicate resistant microorganisms.[4]

In the longer term, the combination of CHX with silver nanoparticles (AgNPs) showed sustained antimicrobial activity, with a mean zone of inhibition of 16.17 mm at 48 and 72 hours. This sustained efficacy is attributed to the gradual release of silver ions (Ag^+), which enhance bacterial membrane disruption and interfere with bacterial metabolism, ultimately causing cell death.[5] However, the combination of CHX with metronidazole, while initially effective, showed a decrease in efficacy over time, possibly due to the rapid dissociation of metronidazole. Similarly, the combination of CHX with calcium hydroxide initially displayed good antimicrobial activity, but its effectiveness decreased due to the depletion of hydroxyl ions.[6] These findings suggest that combining CHX with either ALX or AgNPs could offer a more effective strategy for achieving long-term antimicrobial effects in root canal therapy, thereby improving treatment outcomes.

Conclusion

This study demonstrated that combining chlorhexidine (CHX) with alexidine (ALX) showed the highest initial antimicrobial activity against E. faecalis, while the combination of CHX with silver nanoparticles (AgNPs) provided sustained efficacy. These combinations may enhance the effectiveness of root canal therapy against resistant microorganisms.

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Conflicts of interest

There are no conflicts of interest.

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