ISSN 0973-2063 (online) 0973-8894 (print)

Bioinformation 22(10): 6830-6834 (2026)

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BIOINFORMATION
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BIOMEDICAL INFORMATICS

www.bioinformation.net

Volume 22(10)

Research Article

Received October 1, 2026; Revised October 31, 2026; Accepted October 31, 2026, Published October 31, 2026

DOI: 10.6026/973206300226830

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Edited by Ruby Singh
E-mail: bioinformationruby@gmail.com
Citation: Doshi *et al*. Bioinformation 22(10): 6830-6834 (2026)

Comparing intracanal medicaments and their action against *Enterococcus faecalis*

Meenal Doshi, Nitin Kararia*, Shyam Agrawal, Naveen Chaudhary, Sameeha Khajuria & Wairokpam Devi Digantic

Department of Conservative Dentistry & Endodontics, Nims Dental College & Hospital, Nims University, Jaipur, Rajasthan, India; *Corresponding author

Affiliation URL:
https://nimsuniversity.org/nims-institute-of-dental/

Author contact:
Meenal Doshi - E-mail: doshimeenal6@gmail.com
Nitin Kararia - E-mail: amazingsaggian@gmail.com

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(10): 6830-6834 (2026)
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Shyam Agrawal - E-mail: agrawal.drshyam@gmail.com
Naveen Chaudhary - E-mail: nvnchaudhary@gmail.com
Sameeha Khajuria - E-mail: sameehakhajuria13@gmail.com
Wairokpam Devi Digantic - E-mail: dr.digantic@gmail.com

Abstract:
Complete elimination of microorganisms is essential for endodontic success, but *Enterococcus faecalis* frequently persists in treated canals. Therefore, it is of interest to compare the triple antibiotic paste (TAP), 0.05% octenidine dihydrochloride (OD) and $Ca(OH)_2$ + 2% chlorhexidine against *E. faecalis*. Eighty extracted single-rooted teeth were inoculated for 14 days and randomly assigned to four groups (n=20), with bacterial counts assessed before and after treatment. All experimental groups significantly reduced bacterial counts ($P<0.05$), with TAP showing the greatest reduction (95.74%), followed by $Ca(OH)_2$ + CHX (93.86%) and OD (92.84%). Thus, TAP showed the highest antibacterial efficacy, while $Ca(OH)_2$ + CHX and OD showed substantial activity against *E. faecalis*.

Keywords: Intracanal medicament, triple antibiotic paste (TAP), octenidine dihydrochloride (OD), calcium hydroxide, chlorhexidine, *Enterococcus faecalis*, colony-forming units

Background:
Successful endodontic treatment depends on the effective elimination of microorganisms from the root canal system and the prevention of reinfection **[1]**. Despite advances in instruments, irrigants and techniques, complete disinfection remains difficult because of the complex internal anatomy of the canal, comprising fins, isthmuses, lateral canals and apical ramifications, together with bacterial penetration into dentinal tubules and the buffering action of dentin against antimicrobial agents. These factors allow residual microorganisms to survive and give rise to persistent periapical infection and treatment failure **[2]**. Among the microorganisms associated with failed treatment, *Enterococcus faecalis* is one of the most frequently isolated species. It tolerates extreme pH, withstands nutrient deprivation, adheres to collagen, penetrates deeply into dentinal tubules and can survive as a monoculture within obturated canals **[3]**. Its persistence has been attributed to proton-pump activity, biofilm formation and genetic adaptability and its biofilms act as physical and chemical barriers that reduce the penetration and effectiveness of antimicrobial agents **[4]**. Consequently, mechanical instrumentation and irrigation alone are frequently insufficient and intracanal medicaments are required as an adjunct to eliminate bacteria in inaccessible regions and deep within dentin **[5]**. Triple antibiotic paste (TAP) - a combination of ciprofloxacin, metronidazole and minocycline provides broad-spectrum activity against gram-positive, gram-negative and anaerobic organisms and penetrates deep dentinal tubules and can eliminate *E. faecalis* where calcium hydroxide is limited; however, minocycline-related discoloration, cytotoxicity and the risk of resistance are recognized drawbacks **[6]**. Calcium hydroxide, long regarded as the standard medicament, acts through hydroxyl-ion release and a high alkaline pH, but *E. faecalis* survives alkaline conditions by maintaining intracellular pH homeostasis and dentin buffering further limits its action **[7]**. Combining calcium hydroxide with chlorhexidine, a cationic bisbiguanide with substantivity, broadens the antimicrobial spectrum and improves bacterial reduction relative to calcium hydroxide alone **[8]**. Octenidine dihydrochloride (OD), a bipyridine antiseptic, disrupts bacterial cell membranes and cellular metabolism, acts rapidly, retains activity in the presence of organic matter and carries low cytotoxicity with minimal resistance potential, making it an attractive non-antibiotic alternative **[9]**. As no single medicament satisfies all criteria of an ideal disinfectant, a direct standardized comparison of these three agents against *E. faecalis* is warranted **[10]**. Therefore, it is of interest to compare the antibacterial efficacy using colony-forming-unit (CFU) analysis, an objective and reproducible measure of bacterial reduction.

Materials and Methods:
This comparative *in vitro* experimental study was conducted in the Department of Conservative Dentistry & Endodontics in collaboration with the Department of Microbiology under aseptic conditions, following institutional ethics approval. Eighty freshly extracted single-rooted, single-canalled human permanent teeth with fully formed apices, patent canals and no previous endodontic treatment were included; teeth with cracks, caries extending to the root, resorption, calcified canals or open apices were excluded. Teeth were cleaned, disinfected in 2.5% sodium hypochlorite and stored in 0.9% saline at 4°C. Crowns were decoronated with a diamond disc under water cooling to a standardized root length of 14 mm and access was prepared with a round bur. Working length was established 1 mm short of the apical foramen. Canals were prepared with rotary files to size 25/0.04, irrigated with 2.5% sodium hypochlorite between files, followed by 2 mL of 17% EDTA for smear-layer removal and a final sterile-saline rinse. Canals were dried with paper points, apices sealed with self-cure acrylic resin, external surfaces coated with cavity varnish and specimens autoclaved at 121°C, 15 psi for 20 min. A suspension of *E. faecalis* (ATCC 29212) in brain heart infusion broth was adjusted to 0.5 McFarland ($\approx 1.5 \times 10^8$ CFU/mL); 5 µL was inoculated into each canal, with re-inoculation every 72 h over 14 days at 37 °C to allow deep tubular colonization. Baseline sampling (CFU1) used paper points retained for 60 s, transferred to phosphate-buffered saline and broth, serially ten-fold diluted, plated on bile esculin agar and incubated at 37°C for 24h; characteristic black colonies were counted. Specimens were then randomly allocated (n = 20) to Group I (TAP, 1 mg/mL, mixed 1:1 with distilled water), Group II (0.05% OD gel), Group III $Ca(OH)_2$ mixed with 2%

CHX gel and Group IV (untreated control). Medicaments were placed with an endodontic plugger and specimens incubated for 7 days at 37°C. Medicaments were then removed by saline irrigation and post treatment sampling (CFU2) performed identically. Data (normally distributed; Shapiro–Wilk P > 0.05) were analyzed by one way ANOVA with Bonferroni post-hoc tests using SPSS v23 (P < 0.05).

Results:

Baseline counts were comparable across all groups (mean CFU1 ≈ 1582 × 10^7 CFU/mL), with one-way ANOVA showing no significant difference (P = 0.981, F = 0.034; **Table 1, Figure 1**) and post-hoc comparisons confirmed no significant pairwise differences (all P > 0.05; **Table 2**), verifying uniform initial contamination. After 7 days, CFU2 differed highly significantly among groups (P = 0.001, F = 91211.5; **Table 3, Figure 2**) and every pairwise comparison was significant (P < 0.001; **Table 4**). Group I (TAP) showed the lowest post-treatment count (67.5 × 10^7 CFU/mL), followed by Group III ($Ca(OH)_2$ + CHX; 97.05) and Group II (OD; 113.3), whereas the control remained high (1568.3). Percentage reduction was likewise highly significant (P = 0.001, F = 5341051.5; **Table 5, Figure 3**), with all pairwise comparisons significant (P < 0.001; **Table 6**). TAP achieved the greatest reduction (95.74%), followed by $Ca(OH)_2$ + CHX (93.86%) and OD (92.84%), while the control showed minimal change (0.86%), giving the order TAP > $Ca(OH)_2$ + CHX > OD > control.

Table 1: Intergroup comparison of mean distribution of CFU1 (×10^7 CFU/mL)

Group	N	Mean	SD	SE	95% CI(Lower–Upper)	Min	Max	P value	F value
Group I	20	1583.35	21.58	4.82	1573.25–1593.45	1548	1621	0.981	0.034
Group II	20	1582.5	20.27	4.53	1573.01–1591.99	1549	1615		
Group III	20	1581.3	21.86	4.89	1571.07–1591.53	1547	1618		
Group IV	20	1581.85	21.63	4.84	1571.73–1591.97	1545	1618		
Total	80	1582.25	20.95	2.34	1577.59–1586.91	1545	1621		

Table 2: Intergroup comparison of mean difference of CFU1 (Bonferroni post-hoc analysis)

(I) Group	(J) Group	Mean Diff (I−J)	Std. Error	Sig.	95% CI Lower	95% CI Upper
I	II	0.85	6.749	0.999	−16.880	18.58
I	III	2.05	6.749	0.99	−15.680	19.78
I	IV	1.5	6.749	0.996	−16.230	19.23
II	III	1.2	6.749	0.998	−16.530	18.93
II	IV	0.65	6.749	1	−17.080	18.38
III	IV	−0.550	6.749	1	−18.280	17.18

Groups: I – TAP; II – OD; III – $Ca(OH)_2$ + CHX; IV – Control.

Table 3: Intergroup comparison of mean distribution of CFU2 (×10^7 CFU/mL)

Group	N	Mean	SD	SE	95% CI (Lower–Upper)	Min	Max	P value	F value
Group I	20	67.5	2.56	0.57	66.30–68.70	64	73	0.001	91211.5
Group II	20	113.3	3.21	0.72	111.80–114.80	108	119		
Group III	20	97.05	2.63	0.59	95.82–98.28	93	102		
Group IV	20	1568.3	21.31	4.76	1558.33–1578.27	1532	1604		
Total	80	461.54	643.32	71.93	318.37–604.70	64	1604		

Table 4: Intergroup comparison of mean difference of CFU2 (Bonferroni post-hoc analysis)

(I) Group	(J) Group	Mean Diff (I−J)	Std. Error	Sig.	95% CI Lower	95% CI Upper
I	II	−45.800	3.456	0	−54.879	−36.721
I	III	−29.550	3.456	0	−38.629	−20.471
I	IV	−1500.800	3.456	0	−1509.879	−1491.721
II	III	16.25	3.456	0	7.171	25.329
II	IV	−1455.000	3.456	0	−1464.079	−1445.921
III	IV	−1471.250	3.456	0	−1480.329	−1462.171

**Mean difference significant at the 0.05 level. Groups: I – TAP; II – OD; III – $Ca(OH)_2$ + CHX; IV – Control.*

Table 5: Intergroup comparison of mean distribution of reduction percentage

Group	N	Mean	SD	SE	95% CI (Lower–Upper)	Min	Max	P value	F value
Group I	20	95.74	0.11	0.02	95.69–95.79	95.5	95.91	0.001	5341051.5
Group II	20	92.84	0.11	0.03	92.79–92.89	92.63	93.03		
Group III	20	93.86	0.08	0.02	93.83–93.90	93.7	94		
Group IV	20	0.86	0.03	0.01	0.84–0.87	0.81	0.9		
Total	80	70.83	40.66	4.55	61.78–79.87	0.81	95.91		

Table 6: Intergroup comparison of mean difference of reduction percentage (Bonferroni post-hoc analysis)

(I) Group	(J) Group	Mean Diff (I−J)	Std. Error	Sig.	95%CI Lower	95% CI Upper
I	II	2.897	0.029	0	2.822	2.972
I	III	1.874	0.029	0	1.799	1.949
I	IV	94.881	0.029	0	94.806	94.956
II	III	−1.024	0.029	0	−1.099	−0.949
II	IV	91.984	0.029	0	91.909	92.059

ISSN 0973-2063 (online) 0973-8894 (print)
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III	IV	93.008	0.029	0	92.933	93.083

**Mean difference significant at the 0.05 level. Groups: I – TAP; II – OD; III – $Ca(OH)_2$ + CHX; IV – Control.*

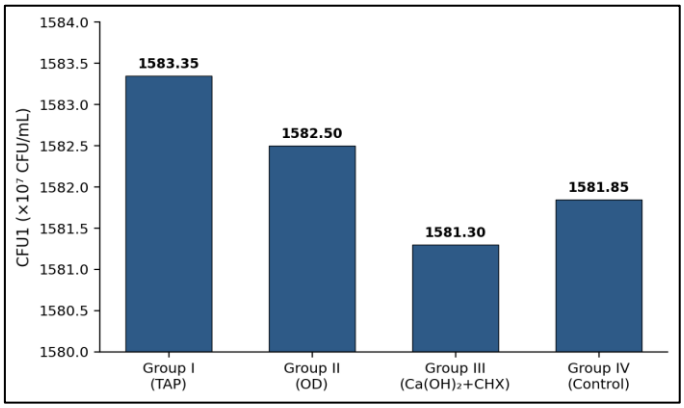


Figure 1: Intergroup comparison of mean distribution of CFU1 (×10^7 CFU/mL)

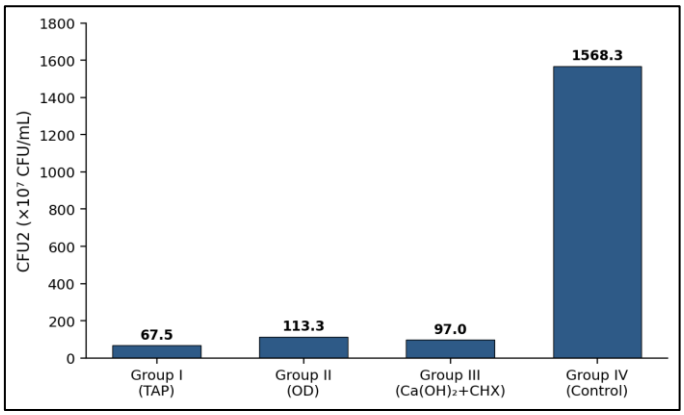


Figure 2: Intergroup comparison of mean distribution of CFU2 (×10^7 CFU/mL)

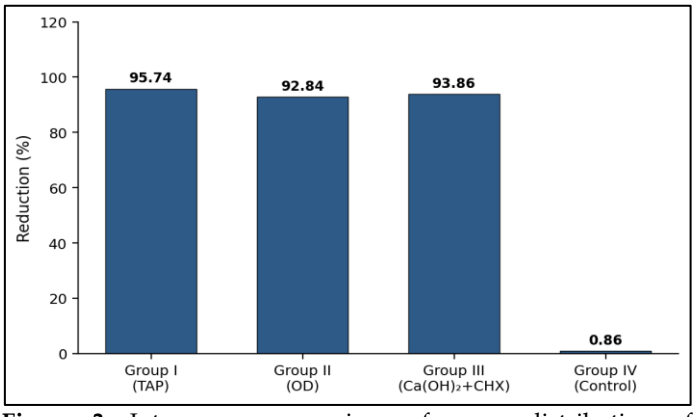


Figure 3: Intergroup comparison of mean distribution of reduction percentage (%)

Discussion:

The comparable baseline counts confirm uniform bacterial distribution, ensuring that post-treatment differences reflect medicament activity rather than variation in initial load. In the present study, TAP produced the greatest bacterial reduction, in agreement with reports that TAP is significantly more effective than calcium hydroxide against *E. faecalis* and yields the highest inhibition among tested medicaments **[10]**. This superiority is attributable to its broad-spectrum, multi-drug composition and its penetration into dentinal tubules, where it acts on biofilm-embedded bacteria. Modifications such as the addition of chitosan or combination formulations further enhance TAP activity, reinforcing the value of multi-agent strategies **[11]**. Nevertheless, complete elimination at all tubular depths was not achieved, indicating that concentration and depth of penetration modulate efficacy **[12]**. $Ca(OH)_2$ + CHX showed moderate, reliable efficacy, consistent with evidence that combining chlorhexidine with calcium hydroxide improves activity relative to calcium hydroxide alone. Calcium hydroxide alone is limited against *E. faecalis* because the organism maintains intracellular pH homeostasis and dentin buffers hydroxyl-ion diffusion **[13, 14]**; chlorhexidine offsets this through membrane disruption and substantivity, providing sustained antibacterial action within dentin. The combination benefits from both the high pH of calcium hydroxide and the prolonged activity of chlorhexidine and in some models approaches the efficacy of TAP **[15]**, although in the present study it remained below TAP owing to the broader spectrum and superior dentinal penetration of the antibiotic paste. Octenidine showed significant but comparatively lower efficacy. Earlier work reported strong, rapid bactericidal action of octenidine in dentinal tubule models and, in some studies, complete elimination of viable cells or superiority to chlorhexidine and calcium hydroxide **[16]**. The lower relative performance here likely reflects the 0.05% concentration used, since higher concentrations and longer contact times improve octenidine efficacy; carrier addition does not consistently enhance its action **[17, 18]**. These findings support octenidine as a promising non-antibiotic agent whose optimal concentration, formulation and application time require further definition. The control group showed the highest counts and negligible reduction, confirming that instrumentation and irrigation alone cannot eliminate *E. faecalis* and underscoring the essential adjunctive role of intracanal medicaments **[19, 20]**. Clinical evidence similarly favors antibiotic based and combination medicaments for resistant infections, while noting that alternatives may still give acceptable outcomes **[21, 22]**. The main limitations are the *in vitro*, single-species design, the fixed medicament concentration and duration, the use of standardized single-rooted teeth and reliance on CFU counting, which may not detect viable but non-culturable organisms; adverse effects such as discoloration and cytotoxicity were not assessed.

Conclusion:

All intracanal medicaments showed antibacterial activity against *Enterococcus faecalis,* with triple antibiotic paste showing the greatest reduction in bacterial count. Calcium hydroxide with 2% chlorhexidine proved to be an effective and biocompatible alternative, while octenidine dihydrochloride showed promising

antimicrobial potential. Further clinical studies are required to validate these findings and optimize intracanal medicament selection.

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