ORIGINAL RESEARCH

Comparative Evaluation of Antimicrobial Efficacy of Calcium Hydroxide, Chlorhexidine, and Triple Antibiotic Paste in Different Combination Forms as Intracanal Medicaments against *Enterococcus faecalis* in Primary Teeth: An *In Vivo* Randomized Clinical Trial

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Abstract

Aim: To compare and evaluate the antimicrobial efficacy against *Enterococcus faecalis (E. faecalis)* between a mix of calcium hydroxide [$Ca(OH)_2$] powder and normal saline, a mix of $Ca(OH)_2$ powder and 2% chlorhexidine (CHX) gluconate solution, a mix of triple antibiotic powder (TAP) and normal saline, and mix of TAP and 2% CHX gluconate solution.

Materials and methods: A total of 60 teeth were included in the study. The first sample (S1) was collected after access opening from the widest canal of the tooth by inserting sterile absorbable paper point no 20 up to the full length of the canal for 1 minute. The second sample (S2) was collected after the chemomechanical preparation and irrigation. After that, subjects were randomly divided into four groups—group I—a mix of $Ca(OH)_2$ and normal saline; group II—a mix of $Ca(OH)_2$ and 2% CHX; group III—a mix of TAP and normal saline; and group IV—a mix of TAP and 2% CHX. Assigned intracanal medicaments were placed in the canals, and the teeth were temporarily sealed with a temporary restorative material. On the 7th day, canals were reopened and irrigated, and a third bacteriological sample (S3) was taken out. Later, canals were filled with suitable obturating material, followed by the placement of the permanent restoration.

Results: There was a very highly significant ($p < 0.005$) difference in *E. faecalis* count in all the groups on day 7 after placement of intracanal medicament, being highest in group IV followed by group II, group III, and group I.

Conclusion: Triple antibiotic powder (TAP) mixed with 2% CHX gluconate solution has superior antimicrobial efficacy against *E. faecalis* in primary teeth.

Keywords: Antimicrobial efficacy, Calcium hydroxide, *Enterococcus faecalis*, Intracanal medicament, Triple antibiotic powder.

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Introduction

Pediatric endodontics is an important fragment of pediatric dentistry and is commonly practiced in dentistry for children. Pulp therapy in primary teeth is an imperious protocol to treat infected pulp. The aim is to keep the deciduous teeth in a viable state till the permanent successor sets in the oral conclave. The biomechanical preparation in pulp treatment in deciduous teeth is kept limited to debridement because of the atypical tortuous character of root canals.[1] Endodontic infection, therefore, embraces a plethora of microorganisms. And thus, the microbial contamination is polymicrobial and is grossly predominated by anaerobes. Furthermost, the microorganisms, such as *Enterococci* and *Streptococci,* are proficiently enduring different conditions of root canal space. Bacterial culture in root canals may display a mixed picture of microbes which include *E. faecalis*, *Streptococci*, *Actinomyces*, *Lactobacilli,* and many more.[2] Out of them, some had shown resistance to endodontic reagents, but specifically, *E. faecalis* is the chief perpetrator in the chart of resilient microorganisms.3 E. *faecalis* is furthermore robust and unaffected by $Ca(OH)_2$ therapy in the root canal habitat.[4]

The biofilm-producing skill of definite endodontic microbes is one of the strategic factors in their pathogenesis. The biofilms' adherence to dentin as well as profound penetration into dentinal tubules, make the process more complex.[5] Hence, the key objective of endodontic management is the complete annihilation of bacterial load from the root canals.[6] The history of pediatric endodontics is being documented with varied antimicrobial chemicals like $Ca(OH)_2$,

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formocresol, CHX, etc. And so, the focal goal line of endodontic treatment is to expunge infection-causing bacteria and their spinoffs from the root canal hoses.[7]

The absolute eradication of bacteria from endodontic canals is not a possibility even after chemo-mechanical preparation though this procedure shows an essential cleaning effect. The disinfection between appointments is a must protocol as the left-out bacteria multiply rapidly during the interappointment time, frequently attaining the zenith pathogenic level as it was notified before starting therapy and here comes the invincible role of antibacterial intracanal dressings.[8,9]

It is well documented that $Ca(OH)_2$ has been considered a panacea for intracanal endodontic infections. As it is alkaline in nature and its pH of 12.5 makes, it easy to dissociate into Ca^{2+} and OH^- ions in an aqueous solution. The OH^- ions release in an aqueous milieu is the prime reason for the antimicrobial activity of $Ca(OH)_2$. These OH^- ions are extremely reactive-free with several biomolecules.[10] This is also one of the very effective medicaments documented in the literature by Evans et al., Basrani et al., and others.[11,12]

Though $Ca(OH)_2$ is always kept at the top-notch position in the list of intracanal medicaments, CHX is also an established name and is an antimicrobial agent with a broad spectrum. The antimicrobial activity of CHX is the result of contact between CHX (positively charged) and bacterial cell walls (negatively charged groups, for example, lipopolysaccharides, phosphate groups, and the carboxyl groups in proteins). The scientific literature in a few studies also points out that CHX gel, when used individually, displays better results than a combination. CHX synergistically adds an antimicrobial effect to other intracanal medicaments. These judgments are in line with conclusions drawn by Gomes et al., and Ghabraei et al.[13,14]

In 1996, Hoshino et al. presented an elusive mixture of TAP in a unique combination with metronidazole and ciprofloxacin with minocycline in the ratio of 1:1:1; they claimed that this antibiotic combination could sufficiently eradicate bacteria from the root canal and promote healing of apical tissue.[15] The outcomes stated by Sato et al. indicated that this triple medicament amalgamation could abolish microorganisms from deep zones of the canal structure.[16] Mozayeni et al. proved that a triple antibiotic mixture could completely impede *E. faecalis* on brain heart infusion (BHI) blood agar medium.[17] The antibiotic mixture is also reported to be rapid in action to expunge near to 99% of *E. faecalis* root canals in just 7 days.[18]

A plethora of literature is available on TAP usage in endodontic therapy of permanent teeth.[18–20] But there is an acute paucity of published literature on this medicament usage in the endodontic therapy of deciduous teeth. In addendum to this, there is an acute deficiency of studies comparing TAP with $Ca(OH)_2$ and CHX for the determination of antimicrobial efficacy. Therefore, this current research was undertaken to compare the antimicrobial efficacy of $Ca(OH)_2$, triple antibiotic medicament, along with saline, or 2% CHX gluconate solution on *E. faecalis* in primary teeth.

Materials and Methods

The research was a randomized clinical trial and was inducted after approval from the Institutional Ethical Committee of Hazaribag College of Dental Sciences & Hospital, Hazaribag, which is affiliated to Vinoba Bhave University of Hazaribag, Jharkhand, India, with reference no HCDSH/ADM/BNF/2019/2436. The clinical research work was completed in the Department of Pedodontics and Preventive Dentistry, Hazaribag College of Dental Sciences & Hospital, Hazaribag, Jharkhand, India.

After assessing the subject's relevant data based on systemic history and drug history, the study groups were divided into four groups of 15 teeth, each as follows:

- Group I: A mix of $Ca(OH)_2$ and normal saline (control group).
- Group II: A mix of $Ca(OH)_2$ and 2% CHX gluconate solution.
- Group III: A mix of TAP and normal saline.
- Group IV: A mix of TAP and 2% CHX gluconate solution.

The study was a single-centered, double-blind, and randomized clinical trial designed for the evaluation of the effects of $Ca(OH)_2$ and triple antibiotic paste against *E. faecalis*.

Subject Selection

A total of 60 subjects between the ages 3–9 years, irrespective of gender, with deciduous mandibular second molar teeth diagnosed with deep dental caries, pulp necrosis or periapical pathosis, or both, clinically, and radiographically having a sufficient crown in subjects who had not taken any antibiotics since 3 months preceding the treatment were incorporated in the study. Those subjects with mobile teeth (Miller's Grade 2 or more), teeth with more than two-thirds of root resorption, and previous endodontic treatment performed teeth were excluded from the study. Medically compromised and uncooperative children were also excluded.

Preparation of TAP

The triple antibiotic mixture contained metronidazole (Sigma Aldrich, India), ciprofloxacin (Sigma Aldrich, India), and minocycline (Sigma Aldrich, India). All three ingredients were mixed in a ratio of 1:1:1 standardized by weight (mg) and volume at the Department of Pharmacology, RG University, Ramgarh, Jharkhand, India. The TAP was then mixed with normal saline or 2% CHX gluconate solution to form a paste as per group, respectively.

Study Design

The participants were examined under standardized settings on the dental chair using diagnostic instruments. Intraoral periapical radiographs were taken for the deciduous mandibular second molar teeth diagnosed with deep dental caries. The clinical and radiographic data were recorded, and the parents of all the subjects were clearly explained about the study. Written informed consent on behalf of participating subjects was received from the parents.

Collection of Microbiological Samples

On the 1st day (day 0), for standardization, all participants received a thorough supragingival scaling using mechanical scalers. The rubber dam isolation was achieved on designated teeth, followed by an access opening with a high-speed air rotor with sterile cooled water, and the pulps were extirpated with broaches. The first sample (S1) was taken out from the widest canal of the tooth by inserting sterile absorbable paper point no 20 up to the canal's full length. The paper points were inserted into the canal for approximately 1 minute. After confirming the working length, instrumentation was done with endodontic H files up to size no 40. After the chemomechanical preparation and irrigation, the second sample (S2) was taken following the same protocol as used for the first sample collection. The enrolled subjects were randomly segregated into four groups, namely group I, group II, group III, and group IV, consisting of 15 teeth each. Assigned medicaments were

carried into the canals with the help of hand plugger no 25 as per group, respectively, after which sterile cotton pellets were placed at orifices of root canals, and the teeth were temporarily packed with temporary restoration (e-Temp, Diadent, Korea). On the 7th day, canals were opened again and irrigated with 2 mL of normal saline to flush out the medicaments and the third bacteriological sample (S3) was taken out. Later, canals were filled with Metapex (Metabiomed, Korea), followed by the placement of stainless-steel crowns (3M ESPE). The samples were placed in sterile Eppendorf vials containing 2 mL of BHI broth. The vials were sealed tightly to avoid contamination and labeled. The labeled vials were kept in a freezer and sent to the laboratory within 1 hour of collection, and samples were assessed for total *E. faecalis* count. The vials were preincubated for 30 minutes at 37°C and were shaken strongly in a vortex apparatus for 60 seconds. Serial 10-fold dilutions were made up to 10^{-6} in 1% sterile normal saline solution. From the dilutions, 0.1–0.2 mL were transported on sterilized sheep blood agar plates (Hi Media Lab, Bengaluru), which were incubated anaerobically at 37°C for 24–48 hours. Microbial colony count was done by the colony counting machine. The identification of microorganisms (*E. faecalis*) was done by Gram staining procedure, catalase production procedure and morphological characteristics of colonies on sheep blood agar using bile esculin agar slants.

Results

Teeth from the subjects were assessed on day 0 and day 7 of the study, and microbiological samples from the widest canal of the teeth were collected on day 0 after access opening (S1) and after instrumentation (S2) and on day 7 post placement of intracanal medicament (S3).

The results for intergroup and intragroup comparison for *E. faecalis* counts were statistically analyzed (Statistical Package for the Social Sciences, version 20); one-way analysis of variance (ANOVA) and Tukey *post hoc* test were used for the statistics with the value of significance $p < 0.05$. On intergroup comparison of the mean count of *E. faecalis* using one-way ANOVA, no significant difference was inferred in *E. faecalis* count in S1 and S2 levels between groups ($p > 0.05$).

On the intergroup assessment of sample S3, a very highly significant difference in the mean *E. faecalis* count (CFU/mL) was observed between all four groups (groups I, II, III, and IV) ($p < 0.005$). Group IV has the least count when compared to other groups, followed by group II, with the highest count in group I (Table 1). On intragroup comparison of mean *E. faecalis* count (CFU/mL) at different levels within all four groups (groups I, II, III, and IV), there was a very highly significant decrease in *E. faecalis* count after instrumentation and after the placement of $Ca(OH)_2$ (Prevest Denpro, India) mixed with normal saline (Lifusion, India) as an intracanal medicament ($p < 0.001$) (Table 2 and 3).

In the present study, results indicated that a mix of TAP and 2% CHX gluconate solution (Prevest Denpro, India) was outstanding against *E. faecalis* when equated with a mix of $Ca(OH)_2$ and normal saline, a mix of $Ca(OH)_2$, and 2% CHX gluconate solution and mix of TAP and normal saline in the elimination of *E. faecalis* in primary molars endodontic canals.

Discussion

The success formula of an endodontic therapy lies in infection riddance and three-dimensional obturation.[21] Majority of endodontic failures are due to incomplete expulsion of microorganisms' load from the canal system leading to the posttreatment periapical lesion. It is without a doubt that residual microorganisms multiply in periods between treatment procedures, recolonize themselves inside a filled root canal conclave, and present as the chief cause of endodontic failures.[22] Thus, the principal endodontic objective should always be to accomplish proper disinfection and preclude reinfection of root canals.

Enterococcus faecalis (E. faecalis), a nonspore cocci, belongs to group IV *Streptococcus* with a negative catalase reduction trait.[23] The history of using intracanal medicaments with antibacterial properties traces back to 1951 when a polyantibiotic paste was first used by Grossman.[24] Subsequently, numerous medicaments were studied and experimented like $Ca(OH)_2$ and others.[25,26] Later on, it surfaced that a blend of different medicaments together could be more synergistic in decontaminating the root canals when compared to medicament used alone. The reason quoted was a dynamic ecosystem of diverse microflora in infected root canals, which is continuously changing, and the possible mutation of bacterial genes existing in canals. Various studies show that $Ca(OH)_2$ alone was unsuccessful in eliminating *Enterococcus* from the root canals.[11,27] The presence of a proton pump in *Enterococcus* maintains the pH homeostasis by pumping protons inside the cell, which reduces the internal pH. Furthermore, buffering nature of root dentin prevents $Ca(OH)_2$ from attaining high alkalinity to kill *Enterococcus*.[3]

On the adjacent side, there are few studies that verify that CHX gel unaccompanied has a superior antimicrobial outcome than in the combination.[13,16] A number of studies have also inferred that if $Ca(OH)_2$ is prepared with 2% CHX gluconate solution, its antimicrobial efficacy is significantly higher than that of the $Ca(OH)_2$ powder mixed with normal saline.[2,28] This shows that CHX synergistically adds an antimicrobial effect to $Ca(OH)_2$.

In our study, a novel preparation, TAP with 2% CHX and its comparison with $Ca(OH)_2$ alone and combination with 2% CHX, was done in terms of *E. faecalis* count inhibition. The statistically significant decrease in *E. faecalis* count was observed in all the groups between S1 and S2 and S2 and S3, which further adds positively to an already proven point that root canal instrumentation in endodontic treatment is capable of reducing *E. faecalis* load to a significant level which goes in agreement with the work done by Bystrom et al., and others in permanent

Table 1: Mean (± SD) *Enterococcus faecalis* count (CFU/mL) in all groups

Groups	*The mean (±SD) E. faecalis count before instrumentation (S1) (CFU/mL)*	*The mean (±SD) E. faecalis count after instrumentation (S2) (CFU/mL)*	*The mean (±SD) E. faecalis count after medication on 7th Day (S3) (CFU/mL)*
I ($N = 15$)	10.35×10^5 ($\pm 0.70 \times 10^5$)	7.28×10^3 ($\pm 0.52 \times 10^3$)	3.43×10^1 ($\pm 0.41 \times 10^1$)
II ($N = 15$)	10.62×10^5 ($\pm 0.58 \times 10^5$)	7.40×10^3 ($\pm 0.60 \times 10^3$)	1.70×10^1 ($\pm 0.49 \times 10^1$)
III ($N = 15$)	10.33×10^5 ($\pm 0.70 \times 10^5$)	10.30×10^3 ($\pm 0.66 \times 10^3$)	2.37×10^1 ($\pm 0.33 \times 10^1$)
IV ($N = 15$)	10.78×10^5 ($\pm 0.67 \times 10^5$)	10.51×10^3 ($\pm 0.61 \times 10^3$)	1.25×10^1 ($\pm 0.16 \times 10^1$)

Table 2: Intergroup comparison of *E. faecalis* count at level S3 using Tukey *post hoc*

Sample level	*Groups*		*Mean difference*	*p-value*
S3	I	II	1.72867	**0.000 (S)**
		III	1.06333	0.000 (S)
		IV	2.17733	0.000 (S)
	II	III	–0.66533	0.000 (S)
		IV	0.44867	0.008 (S)
	III	IV	1.11400	0.000 (S)

$p < 0.05$; S, significant; Tukey *post hoc*

Table 3: Intergroup and intragroup comparison of % change in *E. faecalis* count before and after instrumentation (S1–S2) and after medication (S2–S3)

Groups	*The mean (±SD) E. faecalis count before instrumentation (S1) (CFU/mL) (log_{10})*	*Percentile*	*% change in E. faecalis count after instrumentation and after medication (S1–S2)*	*% change in E. faecalis count after instrumentation and after medication (S2–S3)*
I ($N = 15$)	6.01 (±0.23)	25 Percentile	35.1386	59.3337
		50 Percentile	35.7242	60.6489
		75 Percentile	35.9265	61.4222
II ($N = 15$)	6.03 (±0.24)	25 Percentile	35.5328	66.5532
		50 Percentile	35.8750	68.9342
		75 Percentile	36.1748	70.3499
III ($N = 15$)	6.01 (±0.28)	25 Percentile	35.2681	63.3136
		50 Percentile	35.7783	64.5306
		75 Percentile	36.3011	66.0085
IV ($N = 15$)	6.03 (±0.27)	25 Percentile	35.3494	70.3496
		50 Percentile	35.8422	71.9292
		75 Percentile	36.3200	72.6235
Post hoc pairwise comparison using Tukey's test	Group IV > II > III > I			

teeth.[11,29] Group IV has the least mean *E. faecalis* count (CFU/mL) when compared to other groups, followed by group II, with the highest count in group I. Hence, the results of our study infer that a combination of TAP and 2% CHX gluconate solution was significantly effective in eradicating *E. faecalis* in the root canals more than any other intracanal medicaments, which in the present study were composed of a mix of $Ca(OH)_2$ and normal saline, a mix of $Ca(OH)_2$ and 2% CHX gluconate solution, and mix of TAP and normal saline. The results are congruous with those described by a number of authors *viz*, Evans et al., Schafer et al., Mehta et al., Adl et al., and others.[11,19,30]

Conclusion

- A mix of TAP and 2% CHX gluconate solution is a very potent and effective combination to eliminate *E. faecalis* counts to a significant level in primary teeth.
- Calcium hydroxide [$Ca(OH)_2$] and 2% CHX gluconate combination is also an effective unification to combat *E. faecalis* but less efficacious than TAP and 2% CHX gluconate amalgamation.

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