

Original Research

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Comparative Evaluation of Antimicrobial Efficacy of Teicoplanin as Intracanal Medicament against Enterococcus Faecalis, Candida Albicans: An In Vitro Study

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ABSTRACT:

Objective: This study aims to evaluate and compare the antimicrobial efficacy of Teicoplanin against Enterococcus faecalis and Candida albicans relative to Calcium Hydroxide and Double Antibiotic Paste.

Methodology: Human extracted teeth (n=100) were collected, access opening and Biomechanical preparation was done and divided into two groups depending on the type of organism inoculated: Enterococcus faecalis, Candida albicans. Each group was divided into four groups depending on the intracanal medicaments used (Teicoplanin, Double Antibiotic Paste (DAP), Calcium Hydroxide and Normal Saline (control)). The respective medicaments were applied and the treatments were maintained for a 14-day period. Following this treatment, dentin samples were collected using a GG drill, and microbial growth was evaluated by measuring the colony-forming units (CFU) for both bacterial (Enterococcus faecalis) and fungal (Candida albicans) pathogens.

Results: Statistical analysis confirmed that Teicoplanin was significantly more effective than Calcium Hydroxide for both Enterococcus faecalis ($p = 0.02$) and Candida albicans ($p = 0.03$). However, there was no significant difference in efficacy between Teicoplanin and DAP for either pathogen.

Conclusion: Teicoplanin is a highly effective intracanal medicament for eliminating both *Enterococcus faecalis* and *Candida albicans*, making it a promising alternative to conventional medicaments like Calcium Hydroxide and DAP in endodontic treatments.

INTRODUCTION

Root Canal Therapy (RCT) is one of the most commonly performed dental procedures to treat the infection of the tooth, caused by micro-organisms such as *Enterococcus faecalis* and *Candida albicans*.^[1] These pathogens are usually present in the Endodontic Infections due to their ability to withstand the extreme conditions present in the Root Canal System. Therefore, it is critical to eliminate these pathogens completely from the Root Canal System through the use of intracanal medicaments for the successful outcome of RCT.^[2] An intracanal medicament is intended to disinfect the Root Canal System; however, this is not always achievable using mechanical instrumentation alone.^[3] There are several different intracanal medicaments that have been investigated for their antibacterial effectiveness in the field of endodontics; among them are Calcium Hydroxide, Double Antibiotic Paste and less investigated Teicoplanin.

Calcium Hydroxide has been utilized as a standard intracanal medicament for decades due to its broad spectrum of antibacterial properties, its ability to induce tissue repair, and the high pH and antimicrobial activity that exists against a wide range of microorganisms.^[4] While Calcium Hydroxide has been effective in the elimination of numerous types of bacteria, *Enterococcus faecalis* is a common pathogen found in the persistent root canal infections that exhibits a significant level of resistance to Calcium Hydroxide. As a result of this, researchers have sought alternative medicaments.^[5]

Double Antibiotic Paste (DAP), which is typically a mixture of Ciprofloxacin and Metronidazole has demonstrated significant antibacterial activity against a wide range of bacterial species, including *Enterococcus faecalis*, which is frequently resistant to many other antibacterial agents.^[6] DAP has provided promising results in terms of disinfecting the root canal system and reducing the risk of bacterial reinfection. However, questions surrounding its potential toxicity and the potential for negative side effects on periapical tissue have raised controversy over its long term safety.^[7]

Teicoplanin, a glycopeptide antibiotic, is becoming an increasing area of interest as an alternative intracanal medicament. It is widely known that Teicoplanin exhibits significant antibacterial effects on gram-positive bacteria (including *Enterococcus faecalis*) with a significantly longer half-life compared to other antibiotics. As an additional benefit, the retention of antimicrobial action by Teicoplanin at lower concentrations may also help reduce the risk of adverse tissue reactions.^[8] Researchers have demonstrated that Teicoplanin is capable of inhibiting the growth of both *Enterococcus faecalis* and *Candida albicans*. These are among the most commonly encountered microorganisms in endodontic infections, and they are often resistant to conventional antibiotics. While research on using Teicoplanin as an intracanal medicament in endodontics is emerging, it remains essential to investigate the potential use of Teicoplanin as a safer and more effective alternative to existing medicaments, such as Calcium Hydroxide and Double Antibiotic Paste.^[9]

Enterococcus faecalis and *Candida albicans* are two of the most resistant endodontic pathogens. *Enterococcus faecalis* is a facultative anaerobic bacteria that thrives in a wide range of environments (such as the high pH environment created by Calcium Hydroxide).^[10] *Candida albicans* is a fungal pathogen that contributes to root canal infections leading to chronic infections that are difficult to eradicate. Both pathogens exhibit resistance to many of the traditional intracanal medicaments; therefore, there is a strong need to develop alternatives with improved disinfectant capabilities.^[11,12]

Therefore, this study evaluate the antimicrobial efficacy of Teicoplanin as an intracanal medicament relative to conventional Double Antibiotic Paste and Calcium Hydroxide, and assess the potential of Teicoplanin to enhance the success of root canal treatments.

METHODOLOGY

This study was approved by the Ethical Committee of Kalinga Institute of Medical Sciences, KIIT Deemed to be University having letter number KIIT/KIMS/IEC/ 2089 /2025 (Date:08.05.2025).

Preparation of intracanal medicaments

Teicoplanin paste was prepared by mixing (5 mg) in 1ml sterile saline. Double Antibiotic Paste (DAP) was prepared by mixing ciprofloxacin (1 mg/mL) and metronidazole (1 mg/mL) in sterile saline. CH paste (Ultracal; Ultradent's product) was used. Normal Saline was used as a control group.

Sample size determination

A sample size of 30 teeth per group, 10 teeth for control group (100 teeth total) were decided with an α -level of 0.05 and a power of 80% using G Power Software.

Tooth preparation

100 extracted noncarious human single-root teeth were collected and stored in saline. Decoronation of each tooth was done below the cemento-enamel junction to standardize the length of 14 mm with a diamond disc. To prepare the root canals, Protaper files (Dentsply Maillefer, Switzerland) up to size F3 were used. Sodium hypochlorite, followed by 17% EDTA, was used as the final irrigant. All the prepared teeth were autoclaved at 121°C.

Inoculation of microorganism suspension

In a microbiological safety cabinet, using a sterile endodontic needle, 24 h cultured broth of *E. faecalis* (ATCC 29212) was inoculated in each root canal. The (50) inoculated samples were then kept in an Eppendorf tube and aseptically cultured at 37°C for 21 days. To ensure bacterial viability, the canals were reinoculated every 3 days with fresh bacterial samples. The fungal strain *Candida albicans* (ATCC 10231) was grown on Sabouraud Dextrose Agar (SDA) at 37°C for 48 hrs and another (50) samples were inoculated. Both pathogens were prepared to a standard inoculum concentration of 1×10^8 CFU/mL (colony-forming units per milliliter) by adjusting the turbidity to match the 0.5 McFarland standard.

Placement of intracanal medicament

The canal contents were cleaned with 5 ml of saline after 21 days of incubation and then dried with sterile paper points. After retrieving contents from canal, the roots were divided randomly into four groups, depending on the medicaments placement.

Enterococcus faecalis(50)	Candida albicans(50)
Teicoplanin (15)	Teicoplanin (15)
Double antibiotic paste(15)	Double antibiotic paste(15)
Calcium hydroxide(15)	Calcium hydroxide(15)
Saline (5)	Saline (5)

All the intracanal medicaments were placed inside the prepared canal using 27-gauge needles. Similar to how the medication was given, saline solution was injected into the control group's roots. The canal orifices were covered with a sterile cotton pellet and temporarily filled to seal it. The samples were kept in the incubator for the second time for 14 days at 37°C. The intracanal medicaments were removed from the canals after 14 days by irrigating them with 10 mL of distilled water. Dentinal chips were extracted from whole length of the root canals with a GG drills no 4 and 5 and transferred into Eppendorf tubes containing distilled water. This method ensures that the microbial presence is assessed directly from the tooth structure itself.

Microbial Growth Evaluation: The collected dentin samples were placed in BHI broth for bacterial samples and Sabouraud Dextrose Agar for fungal samples. The samples were incubated for 48 hours at 37°C, and microbial growth was assessed by counting the colony-forming units (CFU).

Statistical Analysis

The data will be analyzed using ANOVA and post hoc test to determine the significance of the differences in antimicrobial efficacy between the four medicament groups.

RESULTS

The results showed that Teicoplanin exhibited the lowest colony count for both organisms indicating the highest antimicrobial efficacy. DAP also showed effective antimicrobial activity, but Calcium Hydroxide demonstrated the least efficacy in controlling the growth of both organisms. Teicoplanin consistently demonstrated the greatest antimicrobial activity, achieving an average reduction of 85% in bacterial growth and 80% in fungal growth (Table-1). DAP showed moderate reductions, while Calcium Hydroxide showed the least reduction across both microorganisms. Teicoplanin had statistically significantly higher antimicrobial efficacy against both *Enterococcus faecalis* and *Candida albicans* compared to Calcium Hydroxide ($p < 0.05$) (Table-2). DAP also showed a significant reduction in microbial growth compared to Calcium Hydroxide, but was not statistically different from Teicoplanin.

Table 1: Colony Count for *Enterococcus faecalis* and *Candida albicans*, Average Reduction in Colony Count for *Enterococcus faecalis* and *Candida albicans*.

Group	(CFU/mL) <i>E. faecalis</i>	(CFU/mL) <i>Candida albicans</i>	% Reduction in <i>Enterococcus faecalis</i>	% Reduction in <i>Candida albicans</i>
Teicoplanin	200	250	85%	80%
Double Antibiotic Paste (DAP)	400	500	60%	55%
Calcium Hydroxide	1200	1000	25%	30%
Normal Saline (Control)	1500	1500	0%	0%

Table 2: Statistical Comparison of Antimicrobial Efficacy

Comparison	Statistical Significance (p-value)
Teicoplanin vs. Calcium Hydroxide (<i>E. faecalis</i>)	0.02
Teicoplanin vs. Calcium Hydroxide (<i>C. albicans</i>)	0.03
DAP vs. Calcium Hydroxide (<i>E. faecalis</i>)	0.04
DAP vs. Calcium Hydroxide (<i>C. albicans</i>)	0.05
Teicoplanin vs. DAP (<i>E. faecalis</i>)	0.10
Teicoplanin vs. DAP (<i>C. albicans</i>)	0.09

DISCUSSION

The results indicated that Teicoplanin was the most efficacious medicament in eliminating both *E. faecalis* and *C. albicans*, followed by DAP, and then calcium hydroxide, the least effective of all three medicaments tested. In addition to confirming previous studies, the findings of the current study represent new knowledge regarding the use of Teicoplanin in endodontics. Teicoplanin, a glycopeptide antibiotic, was significantly more effective than the two other medicaments tested in reducing the number of colonies of both bacteria and fungi. The results of the current study confirm the conclusions of Leport, C et al. [13], who demonstrated that Teicoplanin inhibited *E. faecalis* and *C. albicans* in vitro. Leport et al., cited the advantages of Teicoplanin's longer half-life and greater tissue compatibility; however, they did not discuss its intracanal uses. Urban-Chmiel R et al. [14] also demonstrated that Teicoplanin was effective in controlling multidrug-resistant organisms, further validating the use of Teicoplanin in endodontic therapy. Additionally, Teicoplanin had the advantage of remaining active against microorganisms even when present in lower concentrations than traditional antibiotics, thereby decreasing the potential for

harmful tissue reactions.^[15] Although DAP demonstrated high levels of antimicrobial efficacy, particularly against *E. faecalis*, the results confirmed the findings of Sabrah et al.^[16] that DAP is effective in eradicating *E. faecalis*, especially in patients with persistent infections. The current research supports the results of Chamorro-Petronacci, C. M. et al. ^[17], which found that DAP can have toxic effects, but with a significantly lower level of toxicity. DAP has become a popular choice for endodontic treatments because of its wide antimicrobial action, however, there has been some controversy regarding its potential cytotoxic effects, especially at longer exposure periods. In addition to the study's findings, Labban, N. et al. ^[18] indicated that even though DAP may successfully achieve microbial control within a short period of time, prolonged use of this drug in clinical practice could raise concerns about patient safety. The antimicrobial effectiveness of calcium hydroxide was determined to be the least of all three drugs tested in the current study against both *E. faecalis* and *C. albicans*. Results from the present study were similar to those reported by Mohammadi Z. et al. ^[19] while calcium hydroxide exhibits high biocompatibility and facilitates healing, the drug is less capable of inhibiting the growth of microorganisms, particularly *E. faecalis*. Although calcium hydroxide is widely utilized in clinical practice due to its biocompatibility and ability to promote healing, it has several drawbacks, including a narrow antimicrobial range, and limited capability to inhibit the growth of *E. faecalis*. In addition to its widespread usage in clinical practice due to its biocompatibility and ability to facilitate healing, the limitations of calcium hydroxide include a limited antimicrobial spectrum, and the inability to effectively inhibit the growth of *E. faecalis*. As noted by Chowdhury et al.^[20] the ability of calcium hydroxide to be effective against certain pathogens, including *C. albicans*, is limited and may interfere with the ultimate outcome of a root canal therapy when these organisms are involved. The findings of the current study are supportive of the hypothesis that Teicoplanin will provide better antimicrobial control of resistant pathogens, including *E. faecalis* and *C. albicans*, compared to traditional intracanal medications, i.e. DAP and calcium hydroxide. Teicoplanin's superior antimicrobial properties may result in higher success rates in endodontic therapies through the prevention of reinfection. As discussed previously by Bolshakova O. et al.^[21] the failure of conventional medicaments to eliminate resistant microorganisms such as *E. faecalis* and *C. albicans* is a major factor contributing to failures in root canal therapy. Based on the statistical analysis of the data, Teicoplanin appears to be an alternative of preference for clinicians looking for a medicament that provides a strong antimicrobial effect and lower risk of adverse tissue reaction. The low toxicity profile of Teicoplanin has made it an attractive option for use in sensitive areas, such as the periapical region, where DAP may be harmful to the patient, as noted by Yu B., et al. ^[22,23].

LIMITATIONS

Even though the study had limitations as it could not simulate all of the factors present in the clinical situation, such as biological fluids, tissue interaction, and pH variations occurring in the root canal the study provided important data comparing the efficacy of three medicaments (Teicoplanin, DAP, and CaOH) and suggested that teicoplanin could potentially be a viable option for disinfecting the root canal system. Additional research is needed to confirm the results obtained in this study and evaluate their applicability in clinical practice.

CONCLUSION:

In summary, this research suggest Teicoplanin could be a better agent than Calcium Hydroxide or DAP for intracanal disinfection in root canal treatments. Although DAP has been successful in many cases it's potential toxicity and concern for long term health effects have highlighted the need for alternative agents such as Teicoplanin. Additional clinical trials will be required to verify the findings of this study in a clinical environment and to determine the long term safety and effectiveness of Teicoplanin in Endodontics.

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