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Antimicrobial Efficacy Of Teicoplanin Compared With Triple Antibiotic Paste As An Intracanal Medicament- An In-Vitro Study

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Abstract:
Background*: Persistent intraradicular infection is the primary cause of endodontic failure, with Enterococcus faecalis and Staphylococcus aureus frequently implicated due to their ability to survive within dentinal tubules and resist conventional chemo-mechanical preparation. Although Triple Antibiotic Paste (TAP) is widely used as an intracanal medicament, its association with tooth discoloration, cytotoxicity, and dentin alteration necessitates exploration of safer alternatives. Teicoplanin, a glycopeptide antibiotic effective against Gram-positive organisms, may offer potent antimicrobial activity with fewer adverse effects. Therefore, its potential as an intracanal medicament warrants investigation.*
Materials and Methods*: Seventy-five extracted human single-rooted premolars were prepared, sterilized, and inoculated with clinical isolates of* S. aureus *and* E. faecalis*. Specimens were randomly assigned to three groups: teicoplanin paste, Triple Antibiotic Paste, and distilled water (control). Antimicrobial efficacy was evaluated using colony-forming unit (CFU) counts at baseline, 3rd, 7th, and 14th days. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the Epsilometer (E-test). Data were analyzed using one-way ANOVA and Independent t and Test of significance ($p < 0.05$).*
Results*: Teicoplanin showed a significant reduction in CFU counts against both microorganisms at all time intervals ($p < 0.001$), with complete bacterial elimination by day 14. TAP demonstrated significant antibacterial activity but with residual bacterial presence. Teicoplanin exhibited significantly lower MIC and MBC values compared with TAP ($p < 0.001$). The control group showed a progressive increase in bacterial counts.*
Conclusion: *Teicoplanin demonstrated superior antimicrobial efficacy compared with Triple Antibiotic Paste and may serve as an effective single-antibiotic intracanal medicament in paediatric endodontics.*
Keyword: *Teicoplanin, Triple Antibiotic Paste, Intracanal medicament, Enterococcus faecalis, Staphylococcus aureus.*

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I. Introduction:

Endodontic failure is primarily attributed to the persistence of microorganisms within the root canal system or periapical tissues. Additional contributing factors include inadequate chemo-mechanical debridement, improper obturation, overextension of filling materials beyond the apex, and coronal leakage leading to reinfection.[1,2] Persistent intracanal infection has been strongly associated with periradicular pathosis and treatment failure, as demonstrated by Lin et al. in an analysis of failed endodontic cases.[3] Despite advances in magnification, nickel–titanium instrumentation, and irrigation protocols, predictable success cannot be achieved in all cases.[4]

The adjunctive use of intracanal medicaments following thorough chemo-mechanical preparation has therefore been advocated to enhance disinfection and improve treatment outcomes.[4] A wide range of medicaments has been employed for this purpose.[2] Calcium hydroxide, introduced by Hermann in 1920, remains widely used due to its high alkalinity and antimicrobial activity; however, its limited efficacy against *Enterococcus faecalis*, difficulty of complete removal, and adverse effects on sealer setting reactions restrict its clinical effectiveness.[5,6,7,8,9,10]

Antibiotic-based medicaments such as Triple Antibiotic Paste (TAP), Double Antibiotic Paste, and Ledermix have demonstrated broad antimicrobial activity, including effectiveness against resistant endodontic pathogens.[5,7,11,12,13] However, these materials are associated with significant drawbacks such as tooth discoloration, adverse effects on dentin microhardness, cytotoxicity, and genotoxicity toward periapical and

stem cells.[11,14,15] Given the direct exposure of periapical tissues to intracanal medicaments, their potential cytotoxic effects may compromise healing and regenerative processes.[16]

In light of these limitations, there is a need to explore alternative intracanal medicaments with effective antimicrobial properties and minimal adverse effects. Teicoplanin, a glycopeptide antibiotic isolated from *Actinoplanes teichomyceticus*, exhibits potent bactericidal activity against Gram-positive organisms, including *Enterococcus faecalis* and *Staphylococcus* species, by inhibiting bacterial cell wall synthesis.[17,18,19,20] Its favorable pharmacological profile, including high protein binding, long half-life, and low immunogenicity, makes it a promising candidate for intracanal use.[19]

Therefore, the present study aimed to evaluate teicoplanin as an intracanal medicament by assessing its antimicrobial efficacy and its effect on dentin microhardness.

II. Material And Methods

This in-vitro experimental study was conducted in the Department of Paediatric and Preventive Dentistry, Government Dental College & Hospital, Srinagar, in collaboration with the NIT (National Institute of Technology) Srinagar and Department of Microbiology, Government Medical College & Hospital, Srinagar. Ethical clearance was obtained from the Institutional Ethical Committee (Ref No: GDC/Perio/Ethical Committee/1686; dated 05-01-2024). A total of seventy-five (n = 75) freshly extracted, caries-free, single-rooted human premolar teeth extracted for orthodontic purposes were collected.

Study Design: In-vitro experimental study

Study Location: The research was carried out in the Department of Paediatric & Preventive Dentistry at the Government Dental College & Hospital in Srinagar, in collaboration with the NIT (National Institute of Technology) Srinagar and the Department of Microbiology at the Government Medical College & Hospital Srinagar.

Study Duration: July 2024 to May 2025.

Sample size: 75 extracted teeth

Sample size calculation: Sample size estimation was performed using OpenEpi Version 3, an open-source statistical calculator, for comparison of two independent group means. The calculation was based on the standard deviation of Group 1 ($\sigma_1 = 3.66$) and Group 2 ($\sigma_2 = 3.5$), with an expected difference in group means (Δ) of 3.05. The ratio of sample sizes between the two groups ($k = n_1/n_2$) was maintained at 1, indicating equal allocation. A two-sided confidence level of 95% was considered ($Z_{1-\alpha/2} = 1.96$), and the statistical power was set at 80% ($Z_{1-\beta} = 0.84$). Using these parameters, the required minimum sample size for each group was calculated to ensure adequate power to detect a statistically significant difference between the groups.

Subjects & selection method: Single-rooted human premolar teeth extracted for orthodontic purposes, free of caries, were gathered

The samples were randomly allocated into three groups:
Group 1(25) - Teicoplanin paste was applied.
Group 2(25) - Triple Antibiotic Paste was applied.
Group 3(25)- (Control group) - Distilled water was used without any intracanal medicament.
The groups were further divided for microbiological assessment, dentin microhardness and penetration depth

Inclusion criteria:
1. Single-rooted premolars with a single, straight canal
2. Closed apex
3. Absence of caries or restoration

Exclusion criteria:
1. Teeth with caries, fractures, cracks, or developmental anomalies
2. Open apices, canal calcifications, root resorption
3. Curved roots

Procedure methodology

Teeth were cleaned of debris and soft tissue remnants and stored in distilled water at room temperature. The storage medium was changed every 2–3 days, and samples were used within three months.

Grouping of Samples

The samples were randomly divided into three groups (n = 25 each):

- **Group I:** Teicoplanin paste
- **Group II:** Triple Antibiotic Paste (TAP)
- **Group III (Control):** Distilled water

Root Canal preparation

Crowns were decoronated at the cementoenamel junction to standardize root length to 15 mm.(Figure1,2a,2b) Working length was established at 14 mm using a #15 K-file. Canals were prepared using a rotary NiTi system (NT Gold) in a crown-down manner with irrigation using 5.25% sodium hypochlorite. (Figure 3) A final rinse with 17% EDTA followed by saline was performed. Apical foramina were sealed with composite resin, and root surfaces were coated with nail varnish except at the access cavity.[21] Samples were sterilized using 10% formalin for two weeks.[22]

Bacterial Specimen Collection and Inoculation

Pulp samples were obtained to isolates the *Enterococcus faecalis, Staphylococcus aureus, Streptococcus mitis,* and *Streptococcus salivarius* obtained from failed endodontically treated anterior teeth under strict aseptic conditions.(Figure 4a,4b) To collect pulp samples, a sterile paper point was placed into the canal for 60 seconds, after which it was immediately transferred to a sterile test tube containing 3 mL of Brain Heart Infusion (BHI) broth. The samples were subsequently transported to the microbiology laboratory and incubated at 37°C for 24 hours.[21]

For bacterial inoculation, root samples were aseptically retrieved from the test tubes using sterile tweezers in the Department of Microbiology. The cotton plug and temporary restoration were removed using a sterile spoon excavator. Bacterial suspensions were introduced into the canals using a sterile, spill-free endodontic syringe, following which the access cavities were resealed. The specimens were then transferred to sterile test tubes containing 10 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C for 7 days, with broth replacement after 48 hours.[21]

After incubation, the samples were removed, disinfected externally using an alcohol swab, and cultured on blood agar and Mitis Salivarius agar plates. Blood agar plates were incubated aerobically at 37°C for 24 hours, while Mitis Salivarius agar plates were incubated at 37°C for 48 hours under anaerobic conditions using a candle jar. Microbial identification was performed based on colony morphology, Gram staining, catalase and coagulase tests, bile esculin reaction, and characteristic growth patterns.

Preparation of Intracanal Medicaments[23](Figure 5a,5b,6a,6b)

- **Triple Antibiotic Paste:** Equal proportions of ciprofloxacin (250 mg), metronidazole (400 mg), and tetracycline (100 mg) powders were mixed with 2% propylene glycol.
- **Teicoplanin Paste:** Teicoplanin powder (200 mg) was mixed with 2% propylene glycol to obtain a 2% w/v paste.

MIC Determination (E-Test)

A 0.5 McFarland standard bacterial suspension was lawn-cultured on Mueller–Hinton agar/blood agar plates. E-test strips of teicoplanin and individual antibiotics of TAP were placed on the plates and incubated at 37°C for 24 hours.(Figure 7a,7b) MIC values were recorded at the point of intersection of the inhibition ellipse with the scale on the strip.[1,24,25]

MBC and CFU Determination

Intracanal medicaments were placed using a Lentulo spiral and samples were incubated in BHI broth for 24 hours. Aliquots showing no visible growth were subcultured onto blood agar plates. After incubation, CFUs were counted. MBC was defined as the lowest concentration producing a 99.99% reduction in CFU compared to the initial inoculum.[25,26]

Statistical analysis

The collected data were compiled in a Microsoft Excel spreadsheet (Microsoft, USA). Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). Categorical data were expressed as frequencies and percentages, while continuous data were reported as mean ±

standard deviation (SD). To assess the normality of the data distribution, the Kolmogorov–Smirnov test was employed. Since the data adhered to a normal distribution, Statistical analysis involved the use of parametric tests such as the unpaired t-test and one-way ANOVA. To further assess differences among the groups, Tukey's post-hoc test was performed. For categorical variables, the chi-square test was employed to compare proportions. A p-value below 0.05 was regarded as indicative of statistical significance.

III. Result

COLONY FORMING UNITS

Control Group

In the control group, both *Staphylococcus aureus* and *Enterococcus faecalis* showed a progressive increase in CFU counts from baseline to 14 days.For *S. aureus*, no significant difference was observed between baseline and 3rd day; however, a statistically significant increase in CFU counts was noted from the 7th day onwards ($p<0.05$). Similarly, *E. faecalis* demonstrated a gradual rise in CFU counts, with significant differences observed at later time intervals**,** indicating uninhibited bacterial growth in the absence of antimicrobial agents. (Table1,2) (Figure8,9,10,11)

Triple Antibiotic Paste Group

Triple Antibiotic Paste produced a significant reduction in CFU counts for both microorganisms at all time intervals compared to baseline. For *S. aureus* and *E. faecalis*, a highly significant reduction in bacterial counts was observed as early as the 3rd day, with a continued decrease up to the 14th day ($p < 0.001$). This indicates a strong antimicrobial effect of Triple Antibiotic Paste against both bacterial species. Table (3,4) (Figure 12,13,14,15)

Teicoplanin Group

Teicoplanin demonstrated the maximum antimicrobial efficacy among all groups.

A marked and highly significant reduction in CFU counts of both *S. aureus* and *E. faecalis* was observed from baseline to the 3rd day ($p < 0.001$).

By the 14th day, complete elimination of bacterial growth (0 CFU) was achieved for both organisms, confirming the superior bactericidal activity of Teicoplanin.(Table5, 6) (Figure 16,17,18,19)

Minimum Inhibitory Concentration (MIC)

Teicoplanin exhibited a significantly lower MIC than Triple Antibiotic Paste against both *S. aureus* and *E. faecalis* ($p < 0.001$), indicating greater antibacterial potency. (Table 7,8) (Figure 20,21,22,23,24,25,26)

Minimum Bactericidal Concentration (MBC)

The MBC values for Teicoplanin were significantly lower than those of Triple Antibiotic Paste against both microorganisms ($p < 0.001$), confirming the superior bactericidal activity of Teicoplanin.(Table 9,10)

Table 1- Comparison of Colony-Forming Units (CFU/ml) in Control group against *Staphylococcus aureus* at Different Time Intervals using One Way ANOVA

	Control group	Post-hoc intragroup comparison		
	Intragroup comparison	**Mean±SD**	**Mean difference**	**p value**
Baseline(1929.4 ± 171.65)	3rd day	2007.4 ± 194.75	-78	**p=0.757 NS**
	7th day	2535.53 ± 277.42	-606.13333*	p<0.001**
	14th day	2804.27 ± 206.74	-874.86667*	p<0.001**
3rd Day(2007.4 ± 194.75)	7th day	2535.53 ± 277.42	-528.13333*	p<0.001**
	14th day	2804.27 ± 206.74	-796.86667*	p<0.001**
7th Day(2535.53 ± 277.42)	14th day	2804.27 ± 206.74	-268.73333*	p=0.007*

Table 1 shows the intragroup comparison in Control group against *Staphylococcus aureus* and its comparison at four time intervals. There was a no statistically significant difference in the CFU counts (**p=0.757**) between Baseline and 3rd day. There was a statistically highly significant difference in the CFU counts (**p<0.001)** between Baseline, 7th day& 14th day.

Table 2- Comparison of Colony-Forming Units (CFU/ml) in Control group against *Enterococcus faecalis* at Different Time Intervals using One Way ANOVA

	Control Group	Post-hoc intragroup comparison		
	Intragroup comparison	**Mean ±SD**	**Mean difference**	**p value**
Baseline (1983.73±62.54)	3rd day	2158.47±317.03	-174.733	**p=0.443 NS**
	7th day	2354.47± 415.38	-370.73333*	p=0.012*
	14th day	2627.67 ± 358.85	-643.93333*	p<0.001**

3rd Day (2158.47±317.03)	7th day	2354.47± 415.38	-196	**p=0.341 NS**
	14th day	2627.67 ± 358.85	-469.20000*	p=0.001*
7th Day (2354.47± 415.38)	14th day	2627.67 ± 358.85	-273.2	**p=0.099 NS**

Table 2 shows the intragroup comparison in Control group against *Enterococcus faecalis* and its comparison at four time intervals. There was a statistically significant difference in the CFU counts (**p=0.012**) between Baseline and 7th day. There was a statistically highly significant difference in the CFU counts (**p<0.001)**) between Baseline and 14th day, 3rd Day and 14th Day.

Table 3- Comparison of Colony-Forming Units (CFU/ml) Following Triple Antibiotic Paste Treatment *Staphylococcus aureus* at Different Time Intervals using One Way ANOVA

	Triple Antibiotic Paste	**Post-hoc intragroup comparison**		
Days	**Intragroup comparison**	**Mean +/-SD**	**Mean difference**	**p value**
Baseline(1895.67 ± 114.32)	3rd day	620.33 ± 51.40	1275.33333*	p<0.001**
	7th day	307.80 ± 38.07	1587.86667*	p<0.001**
	14th day	163.33 ±16.56	1732.33333*	p<0.001**
3rd Day(620.33 ± 51.40)	7th day	307.80 ± 38.07	312.53333*	p<0.001**
	14th day	163.33 ±16.56	457.00000*	p<0.001**
7th Day(307.80 ± 38.07)	14th day	163.33 ±16.56	144.466607*	p<0.001**

Table 3 shows the intragroup comparison in Triple Antibiotic Paste against *Staphylococcus aureus* and its comparison at four time intervals. There was a statistical significant difference in the CFU counts (**p < 0.001**) between Baseline and 3rd day, Baseline and 7th day, Baseline and 14th day, 3rd day and 7th day, 3rd day and 14th day, 7thday and 14thday respectively.

Table 4 Comparison of Colony-Forming Units (CFU/ml) Following Triple Antibiotic Paste Treatment against *Enterococcus faecalis* at Different Time Intervals using One Way ANOVA

	Triple Antibiotic Paste	**Post-hoc intragroup comparison**		
	Intragroup comparison	**Mean+/-SD**	**Mean difference**	**p value**
Baseline(1943.53 ± 69.43)	3rd day	630 ± 52.94	1313.53333*	p<0.001**
	7th day	306.47± 25.46	1637.06667*	p<0.001**
	14th day	231.87 ± 15.93	1711.66667*	p<0.001**
3rd Day(630 ± 52.94)	7th day	306.47± 25.46	323.53333*	p<0.001**
	14th day	231.87 ± 15.93	398.53333*	p<0.001**
7th Day(306.47± 25.46)	14th day	231.87 ± 15.93	-74.60000*	p<0.001**

Table 4 shows the intragroup comparison in Triple Antibiotic Paste against *Staphylococcus aureus* and its comparison at four time intervals. There was a statistical significant difference in the CFU counts (**p < 0.001**) between Baseline and 3rd day, Baseline and 7th day, Baseline and 14th day, 3rd day and 7th day, 3rd day and 14th day, 7thday and 14thday respectively.

Table 5 Comparison of *Staphylococcus aureus* counts (CFU/ml) in Teicoplanin group at various time intervals using ANOVA

	Teicoplanin		**Post-hoc intragroup comparison**	
	Intragroup comparison	**Mean +/- SD**	**Mean difference**	**p value**
Baseline (1855.00 +/-130.538)	3rd day	387.27+/-67.37	1467.73333*	p<0.001**
	7th day	18.80+/-4.6	1836.20000*	p<0.001**
	14th day	0.00+/-0.00	1855.00000*	p<0.001**
3rd Day (387.27 +/-67.37	7th day	18.80+/-4.6	368.46667*	p<0.001**
	14th day	0.00+/-0.00	387.26667*	p<0.001**
7th Day (18.80+/-4.6)	14tc day	0.00+/-0.00	18.8	p<0.001**

Table 5 shows intra group comparison in Teicoplanin group against *Staphylococcus aureus* counts and its comparison at various time intervals. There was a statistically significant difference in the CFU counts (p < 0.001) between Baseline and 3rd day, Baseline and 7th day, Baseline and 14th day, 3rd day and 7th day, 3rd day and 14th day, 7thday and 14thday respectively.

Table 6 Comparison of Colony-Forming Units (CFU/ml) Following Teicoplanin Treatment against *Enterococcus faecalis* at Different Time Intervals using One Way ANOVA

	Teicoplanin	**Post-hoc intragroup comparison**		
Days	**Intragroup comparison**	**Mean+/-SD**	**Mean difference**	**p value**
Baseline(1931.60+/-126.73)	3rd day	386.87+/-21.57	1544.73333*	p<0.001**

	7th day	23.73+/-5.42	1907.86667*	p<0.001**
	14th day	0.00+/-0.00	1931.60000*	p<0.001**
3rd day(386.87+/-21.57)	7th day	23.73+/-5.42	363.13333*	p<0.001**
	14th day	0.00+/-0.00	386.86667*	p<0.001**
7th day(0.00+/-0.00)	14th day	0.00+/-0.00	23.73333	p<0.001**

Table 6 shows intra group comparison in Teicoplanin group against *Enterococcus faecalis* counts and its comparison at various time intervals. There was a statistically significant difference in the CFU counts (**p < 0.001**) between Baseline and 3rd day, Baseline and 7th day, Baseline and 14th day, 3rd day and 7th day, 3rd day and 14th day, 7thday and 14thday respectively.

Table 7 Comparison of Minimum Inhibitory Concentration (ug/ml) Between Triple Antibiotic Paste group and Teicoplanin group against *Staphylococcus aureus* using Independent t Test

Inter group comparison	N	Mean ± SD	Mean difference	t value	p value
Triple Antibiotic Paste	15	1.43 ± 0.50	-.86	-5.76	< 0.0001
Teicoplanin	15	0.57±0.29			

Table7 shows the intergroup comparison in Minimum Inhibitory Concentration among Triple Antibiotic Paste and Teicoplanin group against *Staphylococcus aureus*. There was a statistically significant difference between Triple Antibiotic Paste and Teicoplanin group **(p<0.0001).**

Table 8 Comparison of Minimum Inhibitory Concentration(ug/ml) Between Triple Antibiotic Paste and Teicoplanin group against *Enterococcus faecalis* using Independent t Test (µg/ml)

Inter group	N	Mean ± SD	Mean difference	t value	p value
Triple Antibiotic Paste	15	1.685 ±0.47	-1.16	-8.32	< 0.0001
Teicoplanin	15	0.52±0.27			

Table 8 shows the intergroup comparison in Minimum Inhibitory Concentration among Triple Antibiotic Paste and Teicoplanin group against *Enterococcus faecalis.* There was a statistically significant difference between Triple Antibiotic Paste and Teicoplanin group **(p<0.0001).**

Table 9 Comparison of Minimum Bactericidal Concentration for between Teicoplanin and Triple Antibiotic Paste against *Staphylococcus aureus* using Test of Significance (µg/ml)

Group	*Staphylococcus aureus*				
	Mean	SD	Mean difference	t value	p value
Teicoplanin	0.40	0.21	-2.00	-14.142	p<0.001**
Triple Antibiotic Paste	2.40	0.51			

Table 9 shows the intergroup comparison of Minimum Bactericidal Concentration among Teicoplanin, Triple Antibiotic Paste against *Staphylococcus aureus*. There was a statistically significant difference between Teicoplanin and Triple Antibiotic Paste **(p<0.001).**

Table 10 Comparison of Minimum Bactericidal Concentration (µg/ml) for between Teicoplanin and Triple Antibiotic Paste against *Enterococcus faecalis* using Test of Significance

Group	*E. faecalis*				
	Mean	SD	Mean difference	t value	p value
Teicoplanin	0.52	0.27	-2.01	-13.351	p<0.001**
Triple Antibiotic Paste	2.53	0.52			

Table 10 shows the intergroup comparison of Minimum Bactericidal Concentration among Teicoplanin group, Triple antibiotic Paste group against *Enterococcus faecalis*. There was a statistically significant difference between Teicoplanin and Triple Antibiotic Paste (**p<0.001).**

Figure 1- Specimen storage

Figure 1: Extracted premolars stored in distilled water before experimentation.

Figure 2(a,b)- Specimen preparation

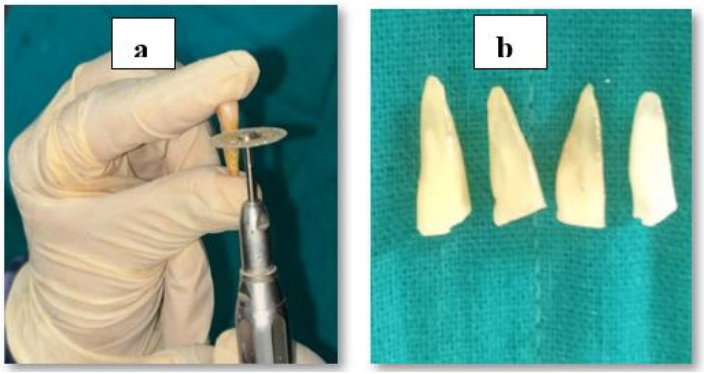

Figure 2a,2b: Decoronation to standardize root length.

Figure 3- Radiographic evaluation

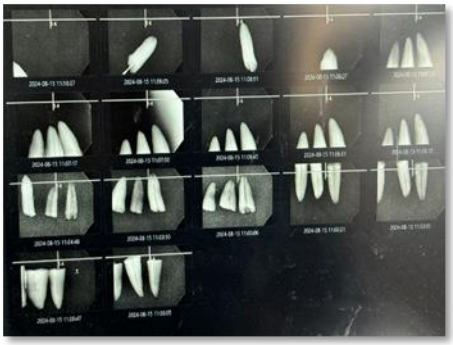

Figure 3: Radiograph to confirm working length.

Figure 4(a,b) Collection of bacterial sample & placement into the BHI broth

Figure 4a,4b: Pulp sample placed into BHI broth.

Figure 5(a,b)- Preparation of Teicoplanin Antibiotic Paste

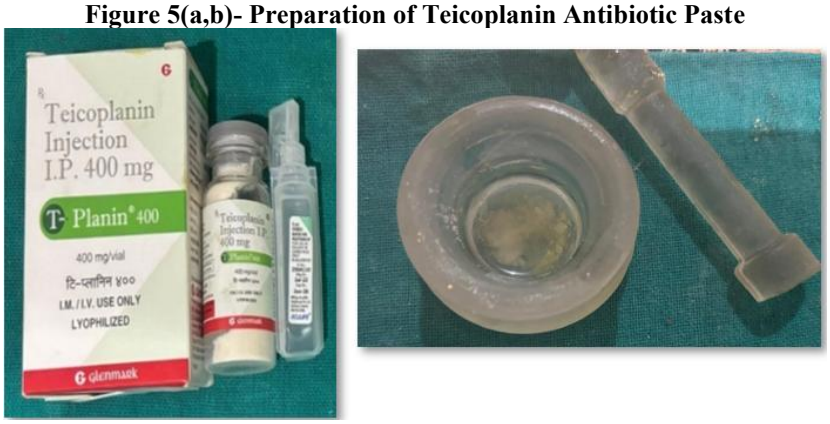

Figure 5a,5b: Preparation of Teicoplanin paste using 2% propylene glycol.

Figure 6(a,b)-Preparation of Triple Antibiotic Paste(Ciprofloxacin, Metronidazole &Tetracycline)

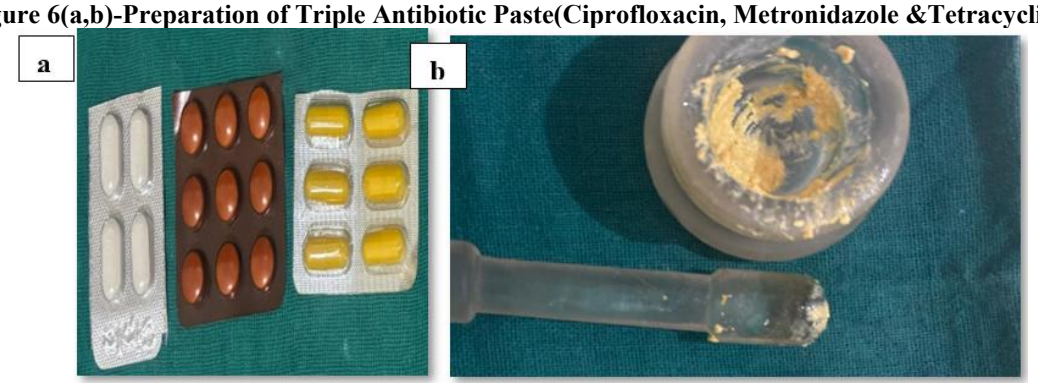

Figure 6a,6b: Preparation of Triple Antibiotic Paste.

Figure 7(a,b)- Bacterial streaking on agar plates

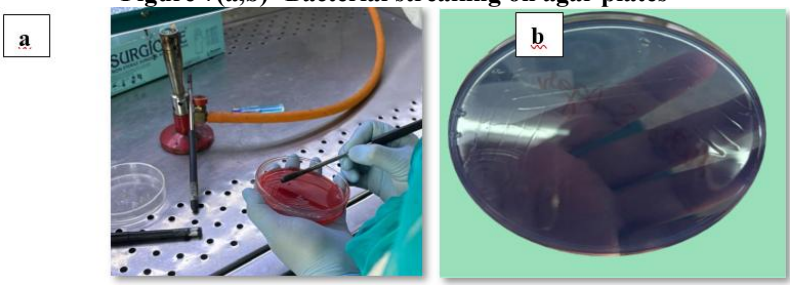

Figure 7a,7b: Streaking of bacterial sample on blood agar and Mitis Salivarius agar

Figure 8- CFU at Baseline of control group

Figure 8: Colony-forming units (CFU) at baseline in the control group prior to medicament application.

Figure 9- CFU at 3rd Day of control group

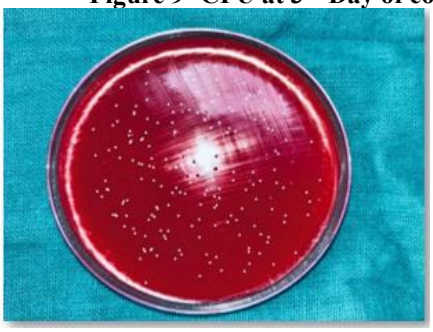

Figure 9: Colony-forming units (CFU) at 3rd day in the control group, showing bacterial growth in the absence of intracanal medicament.

Figure 10- CFU at 7th Day of control group

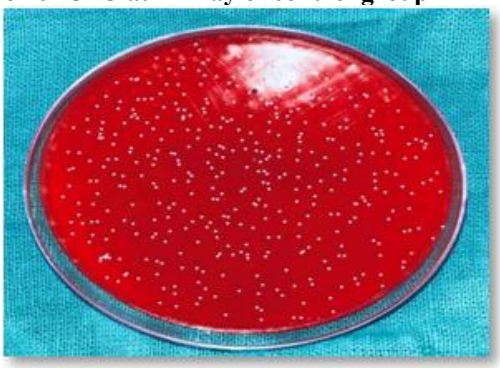

Figure 10: Colony-forming units (CFU) on the 7th day in the control group, demonstrating increased bacterial growth without medicament application.

Figure 11- CFU at 14th Day of control group

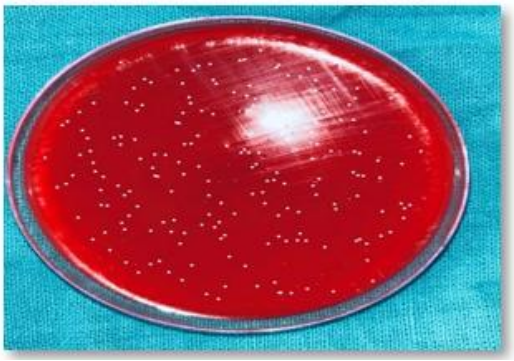

Figure 11: Colony-forming units (CFU) on the 14th day in the control group, showing further progression of bacterial growth.

Figure 12- CFU at Baseline of Triple Antibiotic Paste

Figure 12: Baseline colony-forming units (CFU) in the Triple Antibiotic Paste group prior to medicament placement.

Figure 13- CFU at 3rd Day of Triple Antibiotic Paste

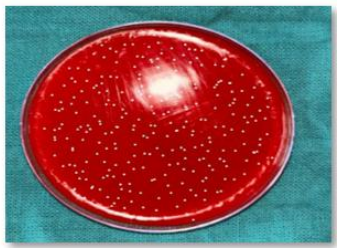

Figure 13: Colony-forming units (CFU) on the 3rd day following application of Triple Antibiotic Paste, showing a marked reduction in bacterial growth.

Figure 14- CFU at 7th Day of Triple Antibiotic Paste

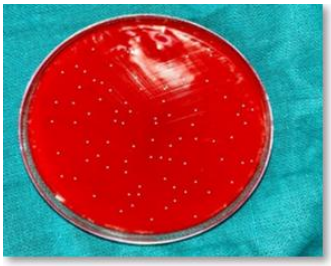

Figure 14: Colony-forming units (CFU) on the 7th day following application of Triple Antibiotic Paste, demonstrating further reduction in bacterial growth.

Figure 15- CFU at 14th Day of Triple Antibiotic Paste

Figure 15: Colony-forming units (CFU) on the 14th day following application of Triple Antibiotic Paste, showing sustained antibacterial effect.

Figure 16- CFU at Baseline with Teicoplanin paste

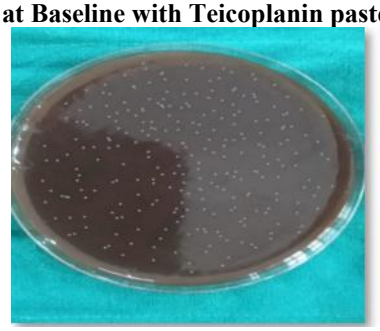

Figure 16: Baseline colony-forming units (CFU) in the Teicoplanin group prior to medicament application.

Figure 17- CFU at 3rd Day with Teicoplanin Paste

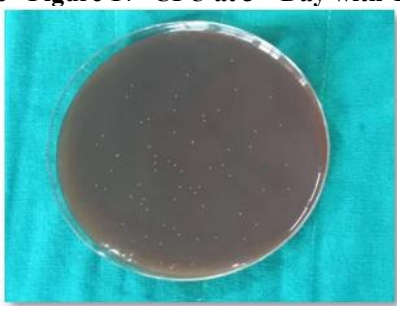

Figure 17: Colony-forming units (CFU) on the 3rd day following application of Teicoplanin paste, showing a significant reduction in bacterial growth.

Figure 18- CFU at 7th Day with Teicoplanin Paste

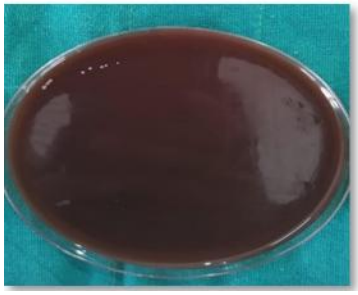

Figure 18: Colony-forming units (CFU) on the 7th day following application of Teicoplanin paste, demonstrating near-complete bacterial reduction.

Figure 19- CFU at 14th Day with Teicoplanin Paste

Figure 19: Colony-forming units (CFU) on the 14th day following application of Teicoplanin paste, showing complete elimination of bacterial growth.

Figure 20- Etests Strip- Teicoplanin

Figure 20: E-test strip for Teicoplanin showing the minimum inhibitory concentration (MIC) against the test organism.

Figure 21- Etests strip- Tetracycline

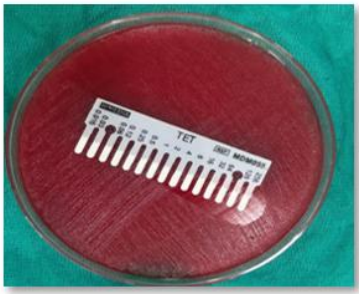

Figure 21: E-test strip for tetracycline showing the minimum inhibitory concentration (MIC) against the test organism.

Figure 22- Etests Strip-Metronidazole

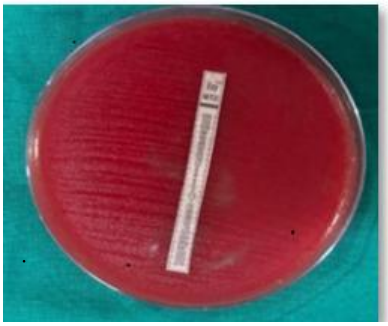

Figure 22: E-test strip for metronidazole showing the minimum inhibitory concentration (MIC) against the test organism

Figure 23- Etests strip- Ciprofloxacin

Figure 23: E-test strip for ciprofloxacin showing the minimum inhibitory concentration (MIC) against the test organism.

Figure 24- Etests Strip- Teicoplanin

Figure 24: E-test strip for Teicoplanin showing minimum inhibitory concentration (MIC) against the test organism.

Figure 25- Etests strip- Tetracycline

Figure 25: E-test strip demonstrating the minimum the inhibitory concentration (MIC) of tetracycline against the test organism.

Figure 26 Etests strip- Metronidazole, Ciprofloxacin

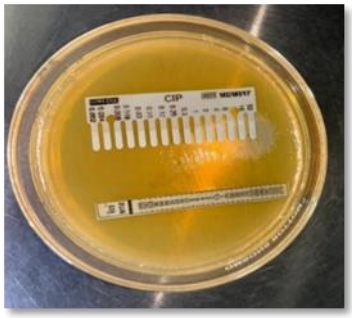

Figure 26: E-test strips indicating resistance pattern, showing high minimum inhibitory concentration (MIC) values for metronidazole and ciprofloxacin against the test organism.

IV. Discussion

Endodontic infections are polymicrobial, predominantly comprising anaerobic bacteria along with facultative species. The infected, nonvital pulp space acts as a protected niche for microorganisms, evading host immune defences and leading to persistent peri radicular inflammation.[27] Successful endodontic therapy depends on effective elimination of these microorganisms, whereas treatment failure is characterized by persistent clinical symptoms and radiographic evidence of pathology.[28]

Persistent intra radicular infection remains the principal cause of endodontic failure, with facultative anaerobes accounting for approximately 85% of isolated microorganisms. Among these, *Enterococcus faecalis* is particularly significant due to its ability to penetrate dentinal tubules, adhere to collagen, form biofilms, and survive harsh environmental conditions.[29,30] These properties make its eradication challenging using conventional chemo-mechanical procedures alone, necessitating the adjunctive use of intracanal medicaments.[30,31]

Calcium hydroxide, despite its widespread use and high alkalinity, has shown limited effectiveness against *E. faecalis*, which can survive prolonged exposure.[9] Additionally, incomplete removal of calcium hydroxide residues compromises sealer adaptation and overall treatment outcomes.[32] These limitations have prompted the use of antibiotic-based intracanal medicaments.

Triple Antibiotic Paste (TAP), comprising metronidazole, ciprofloxacin, and tetracycline, has demonstrated broad antimicrobial efficacy, including against *E. faecalis*.[33] However, its clinical use is limited by drawbacks such as tooth discoloration, cytotoxicity to stem cells of the apical papilla, and the need for higher concentrations to achieve bactericidal effects.[7,34] Double Antibiotic Paste (DAP) was introduced to address discoloration concerns, but it too has been associated with adverse effects on regenerative potential.[34]

Given these limitations, the present study evaluated teicoplanin as a single-agent intracanal medicament. Teicoplanin, a glycopeptide antibiotic structurally related to vancomycin, exhibits potent bactericidal activity against Gram-positive organisms, including *Staphylococcus aureus* and *Enterococcus faecalis*, by inhibiting cell wall synthesis.[35,36] Its prolonged half-life, low toxicity profile, and minimal immunogenicity make it a promising candidate for intracanal application.[87] Furthermore, previous studies have demonstrated its ability to support cell proliferation at clinically relevant concentrations.[37,38]

Methodologically, the use of extracted human single-rooted premolars provided a clinically relevant in vitro model, consistent with previous studies.[39,40] A standardized root canal infection model using clinical isolates and periodic media renewal was employed to closely simulate in vivo conditions and promote biofilm maturation.[29,41] Identification of *E. faecalis* using bile esculin agar ensured high sensitivity and specificity.[42,43] Propylene glycol was selected as the vehicle based on its proven antimicrobial-enhancing and biocompatible properties.[44]

In the present study, the control group showed a significant increase in bacterial counts over time, confirming the absence of inherent antimicrobial activity. In contrast, TAP demonstrated a significant reduction in CFU counts of both *S. aureus* and *E. faecalis*, consistent with earlier reports.[7,45,46] However, teicoplanin showed a more rapid and profound antibacterial effect, with near-complete suppression of both organisms by day 7 and complete elimination by day 14.

Teicoplanin also exhibited significantly lower MIC and MBC values compared to TAP, indicating superior antibacterial potency at lower concentrations. These findings are in agreement with previous studies reporting high susceptibility of *E. faecalis* and *S. aureus* to teicoplanin.[47,48,49,50]

Overall, the results of this study suggest that teicoplanin demonstrates superior antimicrobial efficacy compared to Triple Antibiotic Paste, with effective bactericidal activity at lower concentrations. Its favourable antimicrobial profile and reduced cytotoxic concerns highlight its potential as an alternative intracanal medicament. However, further in vivo and clinical studies are required to validate these findings and assess long-term outcomes.

V. Conclusion

Teicoplanin demonstrated superior antibacterial efficacy and penetration with minimal adverse effect on dentin microhardness compared with Triple Antibiotic Paste. While Triple Antibiotic Paste remained effective, its potential to compromise dentin strength over time is a concern. Therefore, teicoplanin appears to be a more suitable intracanal medicament and warrants further in vivo evaluation.

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