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" Comparative Evaluation Of Probiotics And Triple Antibiotic Paste As Intracanal Medicaments Against Enterococcus Faecalis: An In Vitro Study "

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Abstract:
This study investigates the potential of probiotics as a novel intracanal medicament against Enterococcus faecalis, a common endodontic pathogen, and compares their antibacterial efficacy with a traditional triple antibiotic paste. The study aims to evaluate the viability of probiotics as a potential alternative for root canal treatment. This research seeks to contribute to the development of safer and more effective endodontic therapies.
Materials and Methods*: In this invitro study, the following materials were used: 1. Enterococcus faecalis of ATCC 29212; 2. Probiotics I (VIBACT) 3. Probiotic II (VSL 3) 4. Triple antibiotic paste. Forty-five extracted human permanent mandibular single-rooted pre-molar teeth were collected and stored in distilled water. The specimens were decoronated 1 to 2 mm below the cemento-enamel junction using a safe-sided diamond disk to standardize the root length at 12 mm. The samples were divided into 3 groups of 15 roots each.*
Sample preparation: Human teeth were extracted, decoronated, and standardized. They were then randomly divided into three experimental groups.
Root Canal Treatment: Canals were instrumented using a standardized technique, irrigated with sodium hypochlorite, and treated with EDTA to remove the smear layer. Following this, each canal was inoculated with a standardized concentration of Enterococcus faecalis and sealed.
Medication Application: After a specified incubation period, the canals were rinsed, and the corresponding test medicaments were applied using a standardized technique. The canals were then resealed.
Sample Harvesting: Dentin chips were harvested from the root canals using rotary files. These chips were placed in Brain Heart Yeast (BHY) broth and incubated under controlled conditions.
Optical Density Measurement: The optical density (OD600) of each sample was measured using a spectrophotometer to quantify the concentration of Enterococcus faecalis.
Results*: The results indicate that the medication had a significant effect in Groups I and II but not in Group III. The ANOVA shows significant differences in all three groups after medication, and the Tukey post hoc test confirms that the differences between the groups after medication are statistically significant.*
Conclusion: *This study investigated the antibacterial potential of two commercially available probiotics (VIBACT and VSL 3) and triple antibiotic paste against Enterococcus faecalis. The results demonstrated that both probiotics and the triple antibiotic paste exhibited efficacy in reducing E. faecalis growth. While VSL 3 displayed a larger zone of inhibition compared to VIBACT.*
Keyword: *Antimicrobial; Bacteriotherapy; Bifidobacterium; Endodontics; Innovative; probiotics; triple antibiotic paste.*

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I. Introduction

The success of root canal treatment depends on the three main principles: complete debridement of the root canal system, disinfection, and three-dimensional obturation of the root canal system with a hermetic seal. Even though the root canal procedures are done with the utmost care, certain factors like persistent intra-radicular

and extra-radicular infections, foreign body reactions, and retained cysts lead to failure of root canal treatment. The most commonly associated microorganism in the root canal failed teeth is Enterococcus faecalis, a gram-positive commensal bacterium most commonly extracted from root canals of teeth with persistent endodontic infections.[1,2] Enterococcus faecalis can survive as a single organism without the support of other bacteria inside the root canals, and it can produce a biofilm. This necessitates the introduction of materials that will be able to reduce the number of disease-causing microorganisms in the apical portion of the root canal-filled teeth. One such invention is probiotics.[3]

The most commonly used probiotics in the field of dentistry are of the genus Lactobacillus and Bifidobacterium. These two microorganisms are most abundantly seen in the oral cavity and carious lesions. They can be used as topical applicants. The oral administration of probiotics has been used to reduce plaque and thereby prevent dental caries and periodontal diseases. However, the usage of probiotics as an intracanal medicament in root canal therapy has not been studied extensively. So, the purpose of this research is to evaluate the antibacterial efficacy of probiotics Lactobacillus rhamnosus and Bifidobacterium species over triple antibiotic paste as an intracanal medicament against E. faecalis.[2,4]

II. Material And Methods

The study was carried out in Department of conservative dentistry at Chhatrapati Shahu Maharaj Shikshan Sanstha's Dental College, Chhatrapati Sambhajinagar, Maharashtra

The following materials are used in the present study, which are as follows; 1.Enterococcus faecalis of ATCC 29212, 2.Probiotics I (VIBACT) 3.Probiotic II (VSL 3) 4.Triple antibiotic paste 5.Distilled water. Forty-five extracted human permanent mandibular single-rooted pre-molar teeth were collected and stored in distilled water. The specimens were decoronated 1 to 2 mm below the cemento-enamel junction using a safe- sided diamond disk to standardize the root length at 12 mm. The samples were divided into 3 groups of 15 roots each. (FIG.1)

Group A - Probiotics I (VIBACT)
Group B - Probiotic II (VSL 3)
Group C - Triple antibiotic paste

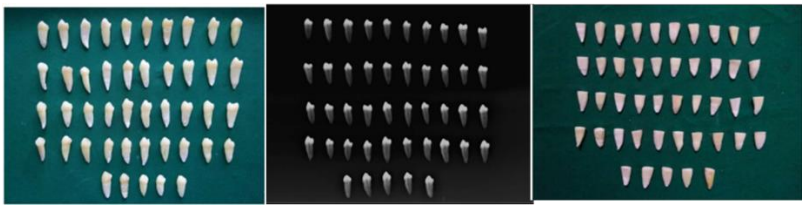


FIG.1

The working length was confirmed using size 10 K-file (Mani, Inc., Japan), and the canal was enlarged using size 15 and 20 K-file, then followed by rotary files used in a sequential manner: size Sx, S1, S2, F1, and F2 Pro-taper Universal (Dentsply Maillefer, Switzerland). 3% sodium hypochlorite as an irrigating solution was used during instrumentation with a sterile 2 ml syringe. (FIG 2)

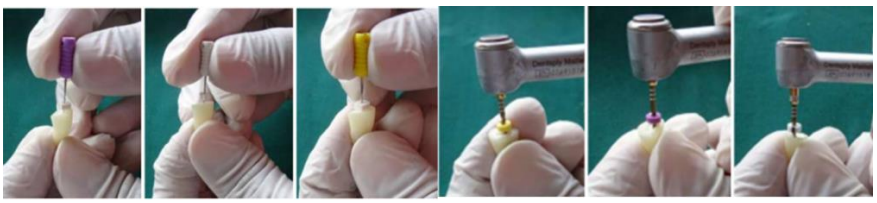


FIG.2

Smear layer removal in the root canal was done using 17% Ethylene Diamine Tetra-acetic Acid (EDTA) solution, which was ultrasonically agitated with U-files size 25. The specimens were autoclaved at 121 °C for 15 minutes. Then three layers of clear nail varnish were applied, covering the entire external root surfaces, and allowed to dry. Bacterial samples of Enterococcus faecalis of the ATCC 29212 strain were used in this study. Each root canal was inoculated with a bacterial solution up to the canal orifice using a sterile syringe. Each canal was then sealed with dental wax, and all samples were incubated in a closed container at 37°C for a period of 7 days.[5] (FIG 3)

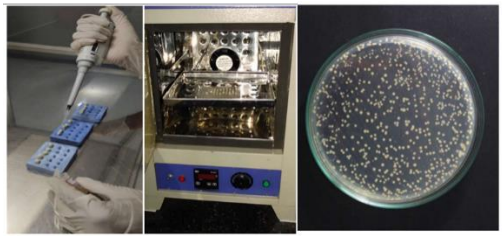


FIG.3

The canals were reinoculated with fresh bacterial samples every 2 days. To check for cell viability and purity of culture, samples were taken from each canal using a sterile paper point (Sure-endo, Korea) and inoculated into a Muller Hilton agar plate and incubated aerobically for 24 hours at 37°C.[6] The canal contents were aspirated, and each canal was rinsed with 5 ml of distilled water using a 5 ml syringe and then dried with sterile paper points (Sure-endo, Korea). The test medicaments were applied to the corresponding groups using a lentulo spiral of 30 sizes. The samples were incubated for 7 days at 37°C after sealing with dental wax. (FIG 4)

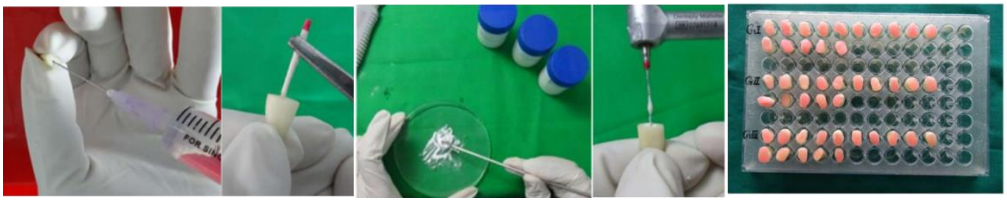


FIG.4

Samples Harvesting Method: The dentin chips from the full length of the radicular dentin were harvested using a sterile rotary nickel-titanium F3 size Pro-Ter universal. The dentin chips were removed from the files by placing them into a sterile Eppendorf tube containing 1.5 ml of Brain Heart Yeast (BHY) broth in a vortex mixture for 30 seconds.[6,7] The files were removed and inspected for dentinal debris remaining in the flutes. If the dentinal debris were present, the files were again placed in a vortex mixer for another 30 seconds for complete removal of dentinal debris. The samples were incubated for 24 hours at 37 °C. Following incubation, each sample was mixed in a vortex mixer for 15 seconds, and 1 ml of solution was pipetted into a cuvette. The optical density 600 (OD600) of each sample was measured to estimate the concentration of Enterococcus faecalis. (FIG. 5)

(FIG. 5)

Statistical analysis

Data obtained was statistically analyzed by One-way analysis of variance (ANOVA) using the SPSS version 2.0, which showed the mean, standard deviation and standard error difference between the group I, group II and group III before and after medication respectively. The values obtained were considered statistically significant as the P value < 0.05. There was statistically significant difference among all the tested groups after medication.

III. Result

Table 1: ANOVA Results Before and After Medication

This table presents descriptive statistics and ANOVA results for three groups both before and after medication.

Three groups (Group I, Group II, and Group III) were analyzed. The mean for each group is provided before and after medication. For example, before medication, Group I had a mean of 0.135, and after medication, it was 0.233.

Standard Deviation (SD) and Standard Error (SE): These give an indication of the variability in the data. The lower the values, the less variation.

ANOVA analysis of variance shows a significant difference in means across groups after medication with a P-value of 0.001, indicating that the differences between groups after medication are statistically significant at the 1% level.

F-value: 136.87, a high value indicating that the variability between the group means is much greater than the variability within the groups.

P-value: For both the "before" and "after" medication scenarios, P-values are significant (below 0.01), meaning the results are not likely due to random chance.

Table 1		N	MEAN	SD	SE	ANOVA	P
Before Medication	Group 1	15	0.135	0.002	0.001	0.313	0.733
	Group 2	15	0.136	0.003	0.001		
	Group 3	15	0.135	0.003	0.001		
	Total	45	0.135	0.003	0		
After Medication	Group 1	15	0.233	0.012	0.003	136.87	0.001
	Group 2	15	0.313	0.044	0.011		
	Group 3	15	0.139	0.02	0.005		
	Total	45	0.228	0.077	0.012		

Table 2: Tukey B Post Hoc Test (After Medication)

This post-hoc test identifies which specific groups differ from one another after medication:

Subset for alpha = 0.05*: Group III (0.139), Group I (0.233), and Group II (0.313) are listed in separate subsets. Since these values fall into different subsets, it indicates that all three groups are statistically significantly different from each other after medication.

Table 2 After Medication				
		Tukey b Post Hoc Test		
Group	N	Subset for Alpha		
		1	2	3
Group 1	15	0.139		
Group 2	15		0.233	
Group 3	15			0.313

Table 3: Paired t-test Results Before and After Medication

This table compares the same groups before and after medication using paired t-tests:

Group I: The difference before and after medication is significant (P = 0.001) with a paired t-value of 31.385, indicating that medication had a significant effect.

Group II: Similar to Group I, the difference is highly significant (P = 0.001), with a t-value of 15.524.

Group III: Unlike the other groups, there is no significant difference in Group III (P = 0.463), meaning the medication did not produce a statistically significant effect for this group.

The results indicate that the medication had a significant effect in Groups I and II, but not in Group III.

The ANOVA shows significant differences in all three groups after medication, and the Tukey post hoc test confirms that the differences between the groups after medication are statistically significant.

Table 3		N	MEAN	SD	SE
Group 1	Before Medication	15	0.135	0.002	0.001
	After Medication	15	0.233	0.012	0.003
Group 2	Before Medication	15	0.136	0.003	0.001
	After Medication	15	0.313	0.044	0.011
Group 3	Before Medication	15	0.135	0.003	0.001
	After Medication	15	0.139	0.02	0.005

The graph 1 shows the optical density before and after medication for three groups (Group I, Group II, and Group III).

Group I: Before medication: mean = 0.135; after medication: mean = 0.233

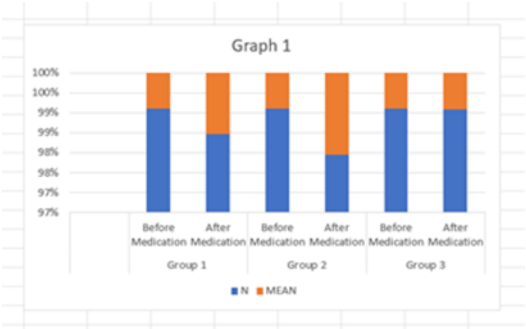
There is a noticeable increase in optical density after medication.

Group II: Before medication: mean = 0.136; after medication: mean = 0.313.

Group II shows the largest increase in optical density after medication, much more significant than Group I.

Group III: Before medication: mean = 0.135; after medication: mean = 0.139.

Group III shows almost no change in optical density after medication, indicating little to no effect of the medication on this group.



Both Group I and Group II show significant increases in optical density after medication, with Group II displaying the highest increase. Group III, on the other hand, shows almost no change, confirming that the medication did not significantly affect this group. It demonstrates that the medication had a significant impact on Groups I and II but not on Group III.

IV. Discussion

Probiotic use has been studied for the treatment of oral health problems. Specifically, the use of probiotics has been explored to aid in the treatment of periodontal problems, halitosis, and caries prevention.8,9 The probiotic approach has not yet been extensively evaluated for use in endodontic therapy. Endodontics is the branch of dentistry that is concerned with the morphology, physiology, and pathology of the human dental pulp and periradicular tissues. It has been established that the primary etiology of endodontic infections is bacteria.[10] Primary infections of the necrotic pulp tissue are generally composed of a mixed bacterial community dominated by anaerobic Gram-negative bacteria. Persistent infections tend to be dominated by a more specific community of bacteria. These bacteria are anaerobic and Gram-positive, in particular E. faecalis.[11,12] Enterococcus faecalis is the most commonly isolated microorganism in failed endodontic cases. Enterococcus faecalis can survive as a single organism without the support of other bacteria, and it can produce a biofilm.[13,14,15] Due to the development of bacterial resistance by microorganisms, the use of antibiotics is being replaced by probiotics. According to Nase et al., the most resistant strain E. faecalis can be eliminated by using probiotics.[16] The probiotics produce various antimicrobial components like organic acids, hydrogen peroxide, bacteriocins, and adhesion inhibitors and thereby inhibit the growth of potentially pathogenic microorganisms.[17,18] The results of the current study are in concordance with the study done by Seifelnasr, who compared five groups of commercial probiotics against E. faecalis and Candida albicans in two phases in vitro. The study results showed that probiotics are effective in preventing the growth of E. faecalis.[19]

The results of the current study comparing the effect of probiotics at 48 hours and 1 week showed a statistically significant difference in the opacity at 48 hours and one week ($p = 0.05$), suggesting sustained action of probiotics against E. faecalis for one week as intracanal medicament.

V. Conclusion

This in vitro study investigated the antibacterial potential of two commercially available probiotics (VIBACT and VSL 3) and triple antibiotic paste against Enterococcus faecalis. The results demonstrated that both probiotics and the triple antibiotic paste exhibited efficacy in reducing E. faecalis growth. While VSL 3 displayed a larger zone of opacity compared to VIBACT in the present study, further investigation is necessary to determine their relative effectiveness in a clinical setting. Further studies with larger sample sizes and diverse bacterial strains are crucial to establishing definitive conclusions about the potential of these probiotics as intracanal medicaments.

References

[1]. Gupta G. Probiotics And Periodontal Health. J Med Life. 2011;4:387–94.
[2]. Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G. Decreased Gum Bleeding And Reduced Gingivitis By The Probiotic Lactobacillus Reuteri. Swedish Dent J. 2006;30:55–60.
[3]. Johal S, Baumgartner JC, Marshall JG. Comparison Of The Antimicrobial Efficacy Of 1.3% Naocl/Biopure MTAD To 5.25% Naocl/15% EDTA For Root Canal Irrigation. J Endod. 2007;33:48–51.
[4]. Allaker RP, Douglas CW. Novel Anti-Microbial Therapies For Dental Plaque-Related Diseases. Int J Antimicrob Agents. 2009;33:8–13.

[5]. Evans M, Davies J K, Sundqvist G, Figdor D. Mechanisms Involved In The Resistance Of Enterococcus Faecalis To Calcium Hydroxide. Int Endod J. 2002;35:221–8.
[6]. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus Faecalis: Its Role In Root Canal Treatment Failure And Current Concepts In Retreatment. J Endod. 2006;32:93–8.
[7]. Nair PNR. Pathogenesis Of Apical Periodontitis And The Causes Of Endodontic Failures. Crit Rev Oral Biol Med. 2004;15:348–8
[8]. Malathi L, Jayasrikrupaa, Balachander N, Vidya Rani, Anbazhagan V, Masthan KMK. Probiotics In Dentistry: A Review. Biosciences Biotechnology Research Asia. 2014;11:193-7.
[9]. Patel R, Dupont HL. New Approaches For Bacteriotherapy: Prebiotics, New-Generation Probiotics, And Synbiotics. Clin Infect Dis. 2015;60(Suppl 2):S108–21.
[10]. Gungor OE, Kirzioglu Z, Kivanc M. Probiotics: Can They Be Used To Improve Oral Health? Benef Microbes. 2015;6:647–56.
[11]. Łysakowska ME, Ciebiada-Adamiec A, Sienkiewicz M, Sokołowski J, Banaszek K. The Cultivable Microbiota Of Primary And Secondary Infected Root Canals, Their Susceptibility To Antibiotics, And Association With The Signs And Symptoms Of Infection. Int Endod J. 2016;49:422–30.
[12]. Carbajal Mejía JB, Aguilar Arrieta A. Reduction Of Viable Enterococcus Faecalis In Human Radicular Dentin Treated With 1% Cetrimide And Conventional Intracanal Medicaments. Dent Traumatol. 2016;32:321–7.
[13]. Siqueira JF, Rocas IN. The Oral Microbiota In Health And Disease: An Overview Of Molecular Findings. Methods Mol Biol. 2016;1537:127–38.
[14]. Jayahari NK, Niranjan NT, Kanaparthy A. The Efficacy Of Passion Fruit Juice As An Endodontic Irrigant Compared With Sodium Hypochlorite Solution: An In Vitro Study. J Investig Clin Dent. 2013;5:154–60.
[15]. Bohora A, Hegde V, Kokate S. Comparison Of Antibacterial Efficacy Of Neem Leaf Extract And 2% Sodium Hypochlorite Against E. Faecalis, C. Albicans, And Mixed Culture—An In Vitro Study. Endodontology. 2010;22:8–12.
[16]. Sinha DJ, Sinha AA. Natural Medicaments In Dentistry. Ayu. 2014;35:113–8.
[17]. Bohora A, Kokate S. Concept Of Probiotics In Endodontics. Int J Adv Res. 2016;4:1137–42.
[18]. Patel HR, Patel RP, Patel MM. Poloxamers: A Pharmaceutical Excipient With Therapeutic Behaviors. Int J Pharmtech Res. 2009;1
[19]. Ravi S, Ananthan HB, Reddy BN, Sankar S, Natarajan SN, Sankar S. Evaluation Of Antibacterial Efficacy Of Two Commercially Available Probiotics As Intracanal Medicines Against Enterococcus Faecalis: An In Vitro Study. J Contemp Dent Pract. 2023 Mar 1;24(3):157-161.