



COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF PROBIOTIC AND CALCIUM HYDROXIDE AGAINST ENTEROCOCCUS FAECALIS AS INTRACANAL MEDICAMENT : AN IN-VIVO STUDY

Dental Science

Dr Geeta I B Rajarajeswari Dental College & Hospital , Bangalore

Dr Ashwini K* Rajarajeswari Dental College & Hospital , Bangalore *Corresponding Author

Dr Sudheer K A Rajarajeswari Dental College & Hospital , Bangalore

ABSTRACT

The principal goal of endodontics is the prevention of periapical infection. E. faecalis is a gram-positive bacterium often isolated in persistent root canal infections. Probiotics are a new potential emerging technology to treat infectious diseases in dentistry and still there are no clear researches about its application in the field of endodontics. The aim of the study was to investigate antibacterial efficacy of Probiotic against E. Faecalis as intracanal medicament and compare it with Calcium hydroxide. 30 single rooted permanent teeth with chronic irreversible pulpitis were selected for the study. They were divided into two groups, Group1: Calcium hydroxide and Group2: Probiotic. Three samples were collected, initial base line sample was taken (S1), after instrumentation second sample was taken (S2) before placement of medicament and then the teeth after one week post medicament sample (S3) was taken. Results: In comparison with calcium hydroxide, probiotic was more effective against E. Faecalis.

KEYWORDS

Calcium hydroxide, Probiotic, Endodontic periapical infection, Enterococcus faecali, Intracanal medicament.

INTRODUCTION

Bacterial violation into the root canals is critical for onset and maintenance of the periapical disease, thus, the principal goal in endodontics is prevention of periapical infection by eliminating microorganisms from the root canal.¹ Chemicomechanical instrumentation and irrigation with an antimicrobial solution alone may not completely eliminate microorganisms from the lateral and accessory root canals, isthmi, and apical deltas.² Thus, intracanal medication between appointments is required to further reduce bacteria in the root canal system.

Enterococcus faecalis is a gram-positive bacterium is frequently encountered in persistent root canal infections. It is highly resistant to bactericidal substances commonly used in endodontic procedures as it can penetrate deep into the dentinal tubules¹ and has the capacity to endure prolonged period of starvation until the availability of adequate nutrition⁵. Its prevalence in periradicular infections ranges from 24% to 77%. In the developing face of dental care, the continued research on E. faecalis and different techniques of its elimination from the root canals may delineate the future of endodontics⁷. Hence E. faecalis was considered in this study.

Calcium hydroxide (CH) is the most commonly used intracanal medicament and is considered to be the gold standard, and the antibacterial property is mainly due to its high pH.² However, CH cannot be considered as a universal intracanal medicament, as it is not equally effective against all bacteria found in the root canal.

Taking into consideration of emerging findings in microbiology, it now seems reasonable that dealing with microbial infection should be to maintain a state of equilibrium within the "Human Microbiome". The "Human Microbiome" is defined as "the recognized, normal microbial component of all humans and animals which is needed for health". Considering that the dentition is a part of the Oral Human Microbiome, it is expected that there should be healthy organisms (probiotics) associated with the teeth in order to enhance endodontic health, since complete sterility is impossible anywhere within the oral cavity.⁴

Simply defined, probiotics are live bacteria that confer a health benefit to the host. The World Health Organization observes probiotics as to be the next most important immune defense system, as the present antibiotics due to their bacterial resistance the use has become limited. Probiotics are a new potential emerging technology to treat infectious diseases in dentistry. they have been applied in the treatment of dental caries and periodontal diseases and has given beneficial results. But, still there are no clear researches about its application in the field of endodontics. The use of Probiotics between visits may enhance this process of elimination. The purpose of this study was to investigate whether probiotic could completely eliminate E. faecalis from the root canal system and compare it with Calcium hydroxide.

MATERIALS AND METHOD OF STUDY

Before data collection, the research protocol was approved by the Research Ethics Board and informed consent from each patient was taken. Subjects were recruited from the patients who had come for the dental treatment to Rajarajeshwari Dental College & Hospital, Bangalore.

30 patients with diagnosis of chronic irreversible pulpitis in single rooted teeth with age range of 15- 50 were selected for the study.

Inclusion/Exclusion criteria

Patients with non-contributory medical history, intact permanent teeth without any previous restoration, with a necrotic or infected pulp as diagnosed clinically and radiographically, with adequate coronal structure for proper isolation, temporization, and restoration were included in the study.

Patients with systemic conditions, acute periapical abscess, retreatment cases, patients on antibiotic therapy within 3 months, teeth with calcified canals, sinus opening, immature apex, internal or external resorption and pregnant women were excluded.

Microbiological samples

Probiotic

Probiotic in the form of commercially available capsule (Providac) was used. It contains Lactobacillus Acidophilus and Bifidobacterium – 1 billion CFU. The capsule was opened and the contents were emptied, mixed with saline to get a paste like consistency to be placed in the canal under sterile conditions.

Calcium hydroxide

Calcium hydroxide powder was mixed with saline to get a paste like consistency to be placed in the canal under sterile conditions.

First treatment session

Each tooth was anaesthetized and isolated with rubber dam, access cavity was prepared using access opening bur 2 and then pulp was extirpated from the canal. # 10 K- file was used inserted into the canal and working length was estimated by an apex locator (Root ZX-J Morita MGF., Japan). Working length was then confirmed using intraoral periapical radiograph (IOPAR). Before instrumentation the first sample (S1) was collected, sterile paper point was inserted into the canal for 30 sec and immediately transferred to the transfer medium. Two paper points were taken for each sample.

Canals were enlarged with K -file till #40 with EDTA-RC prep as the lubricant. Sodium hypochloride and saline were used as irrigants between instrumentation. This was followed by collection of post-instrumentation sample (S2) in the same manner as (S1).

Samples were placed in Appendorf tubes containing transport medium

and transferred to laboratory for processing. After collecting the second sample teeth were divided into 2 groups (15) randomly.

Group 1: calcium hydroxide
Group 2 : probiotic

Canals were dried with paper points followed by a placement of medicament. Then the canals were sealed using Cavit- G and patient was recalled after 7 days.

Second treatment session

After isolation and disinfection, root canals were reinstrumented passively using H- file and irrigated with saline to remove the medicament. Thoroughly cleaned with saline and post medicament sample (S3) was collected same as (S1). Teeth were obturated by lateral compaction method using zinc oxide eugenol sealer and sealed with composite resin.

Microbiological procedure

Samples were provided in the apendorf tubes containing sterile saline, they were stored at room temperature before inoculation. Brain heart infusion agar [Himedia] plates were prepared for inoculation. Samples were directly (100 µL) spread plated on brain heart infusion agar plates and incubated at 37°C for 48hrs. Colony count was determined after 48hrs.

Statistical Analysis:

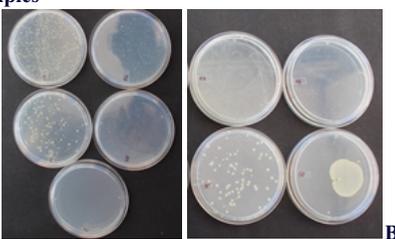
Mann Whitney u Test was used to compare the mean CFUs between 02 groups at different time intervals.

Friedman's Test followed by Wilcoxon Signed Rank Test as post hoc Analysis was used to compare the mean CFUs between different time intervals in each study group. The level of significance [P-Value] was set at P<0.05.

RESULTS

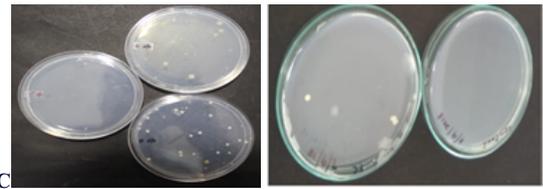
Sl.	Group	Pre	Post	7 Days
1	1	168	118	40
2	1	183	123	16
3	1	157	115	32
4	1	170	131	24
5	1	174	125	30
6	1	179	127	25
7	1	161	131	43
8	1	161	118	37
9	1	174	124	28
10	1	167	136	38
11	1	174	129	16
12	1	161	122	40
13	1	160	115	35
14	1	176	125	29
15	1	159	135	21
16	2	195	164	28
17	2	156	128	34
18	2	169	144	46
19	2	175	152	38
20	2	180	146	35
21	2	163	141	38
22	2	196	157	44
23	2	193	148	42
24	2	172	136	29
25	2	170	129	36
26	2	192	158	27
27	2	151	126	31
28	2	156	130	50
29	2	194	155	41
30	2	195	161	30

Pre test samples



A. Calcium hydroxide B. Probiotic

Post test samples (after 7 days)



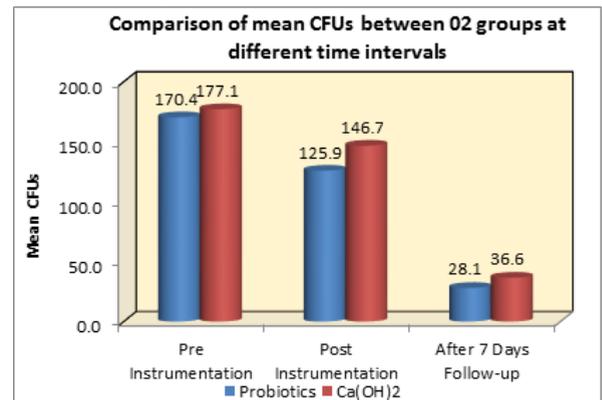
C. Calcium hydroxide D. Probiotic

Time	Group	N	Mean	SD	Mean Diff	P-Value
Pre Instrumentation	Probiotics	15	170.4	10.6	-6.7	0.29
	Ca(OH)2	15	177.1	16.2		
Post Instrumentation	Probiotics	15	125.9	8.4	-20.8	<0.001*
	Ca(OH)2	15	146.7	11.3		
After 7 Days Follow-up	Probiotics	15	28.1	9.5	-8.5	0.02*
	Ca(OH)2	15	36.6	7.0		

* - Statistically Significant

Group	Time	N	Mean	SD	P-Value ^a	Sig. Diff	P-Value ^b
Probiotics	Pre	15	170.4	10.6	<0.001*	T1 Vs T2	0.001*
	Post	15	125.9	8.4		T1 Vs T3	0.001*
	7 Days	15	28.1	9.5		T2 VS T3	0.001*
Ca(OH)2	Pre	15	177.1	16.2	<0.001*	T1 Vs T2	0.001*
	Post	15	146.7	11.3		T1 Vs T3	0.001*
	7 Days	15	36.6	7.0		T2 VS T3	0.001*

* - Statistically Significant



DISCUSSION

The main goal of endodontic treatment is prevention of apical periodontitis. It is the result of constant presence of pathogenic microorganisms in the root canal systems of teeth, and their strength to directly cause acute and chronic inflammation in the periapical tissues⁴. Besides, some authors prefer a single-visit root canal treatment although many studies have shown the importance of intracanal medication between sessions in order to kill microorganisms that chemico-mechanical preparations miss⁵.

Chemico-mechanical preparation done using the instrument and irrigating agents will reduce the bacterial count from the canals but not completely eliminate from the accessory and lateral canals. To see the reduction in the bacterial count, in this study the second sample was taken immediately after instrumentation and the results showed that there was reduction in the bacterial colony count after chemico-mechanical preparation.

E. Faecalis shows high resistance to antibacterial substances, it is capable of producing biofilm which resists destruction from phagocytosis, antibodies and antimicrobials and this bacterium can enter in a viable but nonculturable state during environmental stress. Thus elimination of E.faecalis from the root canal space is the

challenge faced in the endodontic treatment. Therefore, this investigation analyzed the influence of Probiotic and Ca(OH)₂ on the survival of intratubular *E. Faecalis*¹.

In this study calcium hydroxide mixed with saline was used as the intracanal medicament for 7 days and the results showed that there was reduction in the bacterial count. The antibacterial properties of Ca(OH)₂ are attributed to its alkalinity and its ability to destroy the cytoplasmic membrane, denature bacterial proteins, and damage bacterial DNA¹. But in spite of these properties of calcium hydroxide, it is unable to completely eliminate bacteria from the root canals.

There is a need for an innovative or novel approach to the current treatment modalities which can possibly result in a higher, lasting success in endodontic therapy. A promising approach would be to manage endodontic treatment as part of the human microbiome and utilize probiotics in the same manner that they are used for other oral conditions to re-establish equilibrium of healthy flora⁴.

The concept of the "Human Microbiome" has been neglected in the development of new and better strategies used in endodontic therapy including newer intracanal medicaments, even though it is now known to be an integral and undeniable part of human health. The innovative notion in endodontics that the intentional establishment of a microbial equilibrium inside the root canal system by utilizing probiotics, a procedure which might result in improved overall success rates in the reduction of apical periodontitis, needs to be carefully examined and explored.⁴

A study by Hammad (2013) showed that there is no inhibitory effect of lactobacillus on *E. faecalis* whereas another study by Seifelnasr (2014) suggested that probiotics were effective against endodontic pathogens. Limitations of sufficient studies on use of probiotics in endodontics suggest further evaluations for their possible use in treating endodontic infection.

The literature suggests that most commonly used strains are *Lactobacillus* and *Bifidobacterium* genera, commonly found in the oral cavity including caries lesions. These were the first probiotic species to be introduced into research⁵. Hence here the probiotic (*Providac*) used contained lactobacillus and bifidobacterium combinations.

In this study, an innovative approach that might aid in increasing success of endodontic therapy was investigated. This innovative approach involves bacteriotherapy by allowing probiotic organisms to eliminate pathogenic organisms, either by outcompeting / immune modulation or by secreting antimicrobial substances, such as peroxides.

The study showed that probiotic intracanal medicament effectively reduced the bacterial colony count and when compared to the calcium hydroxide, probiotic was more effective in reducing the bacterial count.

Some of the mechanisms of action of these probiotics include the productions of bacteriocin-like inhibitory substances (BLIS) and the altering of the local pH, competing for nutrients, forming physical barriers, and stimulating the immune response.³

Since probiotics are live microorganisms, one likely concern is that they may result in an infection in the host. Several studies have reported that the risk of infection with *Lactobacilli* or *Bifidobacterium* is similar to infections with commensal strains. The risk of infection with these strains is negligible even to those consumers who are immunocompromised (Ouweland, 2003). Probiotics play an important role in combating issues with overuse of antibiotics and antimicrobial resistance. Hence probiotics can be considered as an alternative to the antibiotics.

CONCLUSION

This study evaluated the antimicrobial activity of Calcium hydroxide and Probiotic and found that both were effective against *E. faecalis*. More specifically Probiotic was significantly more effective than Calcium hydroxide. Moreover probiotic is considered to be safe, as it does not cause any side effects and bacterial resistance. It is right time to change the way bacteria are treated in today's new technological era. With fast evolving technology and unification of biophysics with molecular biology, probiotics pose huge opportunity to treat diseases

in a natural and non-invasive way.

ACKNOWLEDGMENTS

The authors deny any conflicts of interest related to this study.

REFERENCES

- Delgado RJ, Gasparoto TH, Sipert CR, Pinheiro CR, Moraes IG, Garcia RB, Bramante CM, Campanelli AP, Bernardini N. Antimicrobial effects of calcium hydroxide and chlorhexidine on *Enterococcus faecalis*. *Journal of endodontics*. 2010 Aug 1;36(8):1389-93.
- Bilgi PS, Shah NC, Mehta J. Comparative Evaluation of Mixture of Calcium Hydroxide and Chlorhexidine, with Triple Antibiotic Paste and Combination of Calcium Hydroxide, Chlorhexidine, and Lycopene on Incidence of Interappointment Flare-up: An in vivo Study. *Int J Clin Dent Res* 2017;1(1):10-14.
- Bohora AA, Kokate SR. Good Bugs vs Bad Bugs: Evaluation of Inhibitory Effect of Selected Probiotics against *Enterococcus faecalis*. *J Contemp Dent Pract* 2017;18(4):312-316.
- Dr. Aarti A Bohora and Dr. Sharad R Kokate. REVIEW ARTICLE : CONCEPT OF PROBIOTICS IN ENDODONTICS. *International Journal of Advanced Research* (2016), Volume 4, Issue 7, 1137-1142
- Charles H. Stuart, Scott A. Schwartz, Thomas J. Beeson and Christopher B. Owatz. *Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment* (*J Endod* 2006; 32:93-98).
- Noordin K, Kamin S. The Effect of probiotic mouthrinse on plaque and gingival inflammation. *Annals of Dentistry University of Malaya*. 2007 Dec 31;14(1):19-25.
- Vivekananda MR, Vandana KL, Bhat KG. Effect of the probiotic *Lactobacilli reuteri* (*Prodentis*) in the management of periodontal disease: a preliminary randomized clinical trial. *Journal of oral microbiology*. 2010 Jan 1;2(1):5344.
- Awadhesh kumar singh. Oral probiotics: the beneficial microbes for dental & periodontal health . *DHR International Journal Of Medical Sciences (DHR-IJMS)* ISSN: 2278-831X, Vol. 3(1), 2012
- Narwal A (2011) Probiotics in Dentistry – A Review. *J Nutr Food Sci* 1:114. doi:10.4172/2155-9600.1000114
- Siqueira Jr JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review (Review). *International Endodontic Journal*, 32, 361±369, 1999.
- Roy H, Stevens, DDS, MS, and Louis I. Grossman, DDS, Dr. reed. dent. Evaluation of the Antimicrobial Potential of Calcium Hydroxide as an Intracanal Medicament. *JOURNAL OF ENDODONTICS*. VOL. 9, NO. 9, SEPTEMBER 1983
- Anusha RL, Umar D, Basher B, Baroudi K. The magic of magic bugs in oral cavity: Probiotics. *J Adv Pharm Technol Res* 2015;6:43-7.
- Zahradnik RT, Magnusson I, Walker C, McDonnell E, Hillman CH, Hillman JD. Preliminary assessment of safety and effectiveness in humans of ProBiora3™, a probiotic mouthwash. *Journal of applied microbiology*. 2009 Aug 1;107(2):682-90.
- Lima RK, Guerreiro Tanomaru JM, Faria Júnior NB, Tanomaru Filho M. Effectiveness of calcium hydroxide based intracanal medicaments against *Enterococcus faecalis*. *International Endodontic Journal*. 2012 Apr;45(4):311-6.
- Turk BT, Sen BH, Ozturk T. In vitro antimicrobial activity of calcium hydroxide mixed with different vehicles against *Enterococcus faecalis* and *Candida albicans*. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2009 Aug 1;108(2):297-301.
- Lana PE, Scelza MF, Silva LE, Mattos-Guaraldi AL, Hirata Júnior R. Antimicrobial activity of calcium hydroxide pastes on *Enterococcus faecalis* cultivated in root canal systems. *Brazilian dental journal*. 2009;20(1):32-6.
- Sathorn C, Parashos P, Messer H. Australian endodontists' perceptions of single and multiple visit root canal treatment. *International endodontic journal*. 2009 Sep;42(9):811-8.
- Sinesi A, Fanuli M, Viganò L, Casu C. *Biointerface Research in Applied Chemistry*.
- Ponce JB, Midena RZ, Pinke KH, Weckwerth PH, Andrade FB, Lara VS. In vitro treatment of *Enterococcus faecalis* with calcium hydroxide impairs phagocytosis by human macrophages. *Acta Odontologica Scandinavica*. 2018 Dec 7:1-6.
- Harini PM, Aneundi RT. Efficacy of a probiotic and chlorhexidine mouth rinses: A short-term clinical study. *Journal of Indian society of pedodontics and preventive dentistry*. 2010 Jul 1;28(3):179.
- Nadkerny PV, Ravishankar PL, Pramod V, Agarwal LA, Bhandari S. A comparative evaluation of the efficacy of probiotic and chlorhexidine mouthrinses on clinical inflammatory parameters of gingivitis: A randomized controlled clinical study. *Journal of Indian Society of Periodontology*. 2015 Nov;19(6):633.
- Rôças IN, Siqueira Jr JF, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *Journal of endodontics*. 2004 May 1;30(5):315-20.
- Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *International Endodontic Journal*. 2002 Mar;35(3):221-8.
- Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Critical Reviews in Oral Biology & Medicine*. 2004 Sep;15(5):308-20.