

Evaluation of the Antibacterial Efficiency Of Intracanal Medicaments On Enterococcus Faecalis: An In-Vitro Study

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ABSTRACT

Aim: To evaluate the antibacterial efficiency of 2%Chlorhexidine gel, Metapex, Calcium hydroxide powder mixed with 2% chlorhexidine solution and Calcium hydroxide paste as intracanal medicaments against E.faecalis bacteria.

Materials and Method: 80 single rooted human mandibular premolar teeth were collected. All the teeth were decoronated and biomechanical preparation was completed. Root canal space of all the teeth were infected with pure culture of E.faecalis bacteria and were randomly into four groups with 20 teeth per each group. Group A: 2%Chlorhexidine gel, Group B: Metapex, Group C: Ca(OH)₂ powder mixed with 2% chlorhexidine solution, Group D: Calcium hydroxide paste, were used as intracanal medicaments. Lentulo spiral instruments were used to place the intracanal medicaments in the root canal space and the teeth were temporarily restored for 7 days. Later, intracanal medicaments were completely removed using canalbrush and root canal space were dried. Dry sterile paper points were used for culture tests from all samples and the number of CFU were counted.

Results: Statistical analysis by One-Way ANOVA showed significant difference in the antibacterial efficiency between the four groups. P-Value is =0.0001.

Conclusion: 2% Chlorhexidine gel showed the highest antibacterial efficiency compared to other intracanal medicaments used in this study.

Keywords: Enterococcus faecalis, Lentulospiral, Canalbrush, Intracanal medicaments.

INTRODUCTION

The primary goal of endodontic therapy is the reduction or elimination of micro-organisms and their by-products from the root canal system. However with proceeding infection, necrosis and apical periodontitis, the entire root canal system becomes invaded by microorganisms. The goal of

endodontic treatment is to kill all microorganisms in the root canal.¹ Enterococcus faecalis is a facultative anaerobic bacteria present in small proportions of the polymicrobial flora in the infected root canals. E. faecalis has an ability to survive harsh environments within the root canal. The prevalence of E. faecalis in primary endodontic infections is 40% and in persistent endodontic

Received: Oct. 11, 2016: Accepted: Nov. 18, 2016

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infections it is 24% to 77%. It binds to dentin and proficiently invades dentinal tubules of the tooth.^{2,3}

Successful root canal treatment depends on three main phases, biomechanical preparation of the root canal, complete disinfection of the root canal space and 3-dimensional obturation of the root canal. Intracanal medicaments are the medicaments placed within the root canal space for a specific number of days, which helps in the total disinfection of the root canal space and it plays a vital role in the success of root canal treatment.^{4,5} 2% Chlorhexidine gel is a broad spectrum antimicrobial agent and it also has a property of substantivity. Metapex is an intracanal medicament in which, the synergistic effect of OH⁻ ions and iodoform overcomes the buffering capacity of dentin and shows excellent antimicrobial action. Calcium hydroxide paste in premixed form is a water based, radiopaque intracanal medicament which acts by diffusion of hydroxyl ions through the dentin.^{6,7,8}

The purpose of this in-vitro study was to evaluate the antibacterial efficiency of 2% chlorhexidine gel, metapex, calcium hydroxide powder mixed with 2% chlorhexidine solution and calcium hydroxide paste in premixed form as intracanal medicaments against enterococcus faecalis bacteria.

MATERIALS AND METHOD

Eighty extracted human permanent mandibular single rooted premolar teeth were collected from the Department of Oral and Maxillofacial surgery, Triveni institute of dental sciences, hospital and research center. Bodri, Bilaspur, Chhattisgarh.

Inclusion criteria: Non-carious, non-fractured, matured with closed root apices, non-restored, single rooted teeth with Vertucci class-I root canal configuration.

Exclusion criteria: Carious, fractured, restored, multirrooted, open apex, previously endodontically treated teeth.

Tooth specimens were cleaned off superficial debris, calculus, tissue tags and stored in 0.1% thymol at room temperature until used. All the specimens were decoronated at cemento-enamel

junction with a diamond disc (DFS, Germany) to obtain a standardized root length of approximately 12 mm for uniform specimens. Pulp tissue was extirpated from all the teeth and working length was visually established with a K-file No. 15 (Mani, Inc, Japan) inserted into the root canal until its tip reached the apical foramen and working length was determined 1 mm shorter to the apical foramen.

All the specimens were vertically mounted in putty blocks (Affinis, Coltene/Whaledent, Inc, OH, USA) to make both handling and identification easier. Biomechanical preparation was done in all the teeth in crown-down technique using Protaper hand files (Dentsply, Maillefer, Switzerland) and the canals were enlarged to an apical size of No. F3 file. During the biomechanical preparation 17% EDTA (Ethylenediaminetetraacetic acid) (Prime Dental Products, PVT, Ltd, Thane, India), 3% sodium hypochlorite (Neelkanth Health Care (P) Ltd, Jodhpur, India) and saline were used as root canal irrigants. All the teeth were then autoclaved at 121°C of temperature at 15 lbs of pressure for 20 minutes to ensure complete sterilization of root canal space. Subsequent to sterilization, all specimens were handled under strict aseptic conditions. Pure culture of *Enterococcus faecalis* bacteria (ATCC 29212, Kwik Stik, Microbiologics, France) (Figure 1) grown in brain heart infusion broth (HiMedia Laboratories, Pvt. Ltd, Nashik, India) which had its optical density adjusted to approximately 1×10^8 CFU/ml by comparing its turbidity to McFarland 0.5 BaSO₄ standard was used to contaminate the root canal space of teeth. The culture was checked for purity by Gram stain and colony morphology.



Fig 1: Enterococcus faecalis bacteria(ATCC 29212)

An inoculum of 10 µlit culture suspension of E.faecalis was suspended into each prepared root canal space of all the teeth using a sterile micropipette (10 µl, liquid handling instrument, Labtop, India) in a laminar air flow cabinet to prevent any airborne contamination and the teeth were incubated aerobically at 37°C of temperature for 48 hours. All the teeth were randomly divided into four groups with 20 teeth per each group. Intracanal medicaments were placed in the root canal space of all the teeth as per their respective groups.

In Group A: 2% Chlorhexidine gel(Cavisept, Neelkanth Health Care, India), Group B: Metapex(Meta Biomed Co.Ltd, Korea), Group C: Calcium hydroxide powder(Safe plus, Neelkanth Health Care, India) was mixed with 2% Chlorhexidine gluconate solution(Asep-Rc, Anabond Stedman Pharma Research Pvt. Ltd, India) into a freshly made paste form, Group D: Calcium hydroxide paste (Premixed form) (Rc Cal Prime Dental Products Pvt Ltd) were used as intracanal medicaments.(Figure 2)

Intracanal medicaments were placed using lentulo-spiral No:25 (Mani, Inc, Prime Dental Products) engine driven instruments rotated slowly at 1000 rpm into the root canal space 1mm shorter to the final working length determined until the medicaments were clinically visible at the root canal orifice.

Small sterile cotton pellets were placed into the coronal access of all teeth and were then temporarily restored with Cavit G(3M, ESPE, Germany) temporary restorative material and were incubated aerobically at 37° C in 100% relative humidity for seven days.

Later, temporary restorations and cotton pellets were removed from all the samples. Intracanal medicaments were completely removed from the root canal space of all the teeth using medium-size canalbrush (Roeko canal brush, Coltene Whaledent) in a slow speed hand piece rotating at 600rpm in a circumferential motion for 30 seconds, upto 1mm shorter to the final working length determined along with the use of 2ml of normal saline per each tooth.

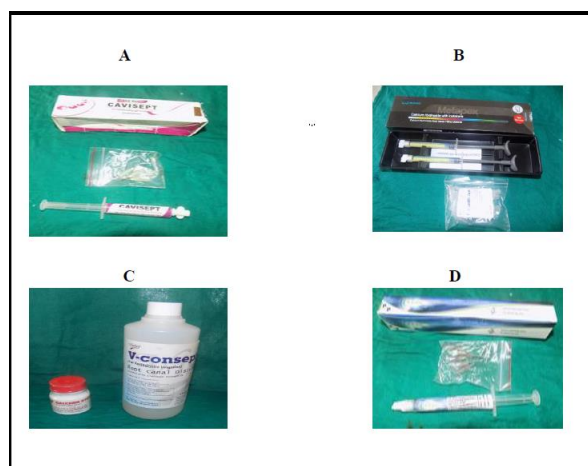


Fig 2: Intracanal medicaments used in the study. 2(A): 2% Chlorhexidine gel, 2(B): Metapex, 2(C): Calcium hydroxide powder was mixed with 2% Chlorhexidine gluconate solution, 2(D): Calcium hydroxide paste (Premixed form).

After accomplishing the removal of excess moisture from the root canals using sterile paper points (DiaDent Group International, Korea), a dry sterile paper point of ISO No.30 was placed into the root canal space of all the teeth to the full working length, left for 10 seconds and the paper points were transferred into the sterile test tubes containing 2ml of PBS (Phosphate Buffered Saline) and vortexed for 30 seconds. Using a inoculation loop (Nicrome wire loop holder) bacterial suspensions from each test tube samples were placed in 5% sheep blood agar plates (HiMedia Laboratories, Pvt. Ltd, Nashik, India). The agar plates were incubated aerobically at 37 degree C for 48 hours. The entire procedure was carried out in strict aseptic conditions. The cultured plates were then evaluated to confirm the growth of E.faecalis bacteria and colony forming units(CFU) were counted using digital colony counter (SECOR, Scientific Engineering Corp, India).

RESULTS

The results were tabulated and statistically analysed (Table: I and Graph: I) with computer software; Statistical Package for Social Sciences(SPSS) Version 16, Using Analysis of variance(One Way ANOVA) and Tukey's - Post Hoc test. One-Way Analysis of Variance is to test the equality of three or more means at one time by

using variances. In our study, One way ANOVA showed highly significant difference in the mean values of Colony Forming Units [CFU] between the four groups ($P=0.0001$). P -Value is = 0.0001, is considered to be highly significant.

According to One Way ANOVA between the four groups, Group A in which 2% Chlorhexidine gel was used as intracanal medicament exhibited minimal growth of enterococcus faecalis bacterial colonies in the culture plates with a mean of 27.00(Figure 3), followed by Group B with a mean of 66.00, Group C with a mean of 68.00 and the maximum growth of the *E. Faecalis* bacterial colonies were seen in Group D with a mean of 105.0(Figure 4), in which Calcium hydroxide paste was used as intracanal medicament. To find exactly which group differs from the other. Tukey's - Post Hoc test was done for inter-group comparison of the four groups. It was found that there was no statistically significant difference in the antibacterial efficiency between Group B and Group C.

The obtained results showed, Group A in which 2% Chlorhexidine gel was used as intracanal medicament exhibited statistically significant higher antibacterial efficacy against *E.faecalis* compared to other intracanal medicaments used in our study. Group C, in which Calcium hydroxide powder was mixed with 2% Chlorhexidine gluconate solution into freshly made paste form and Group D, in which Calcium hydroxide paste in premixed form were used as intracanal medicaments showed least antibacterial efficiency against *E faecalis*.

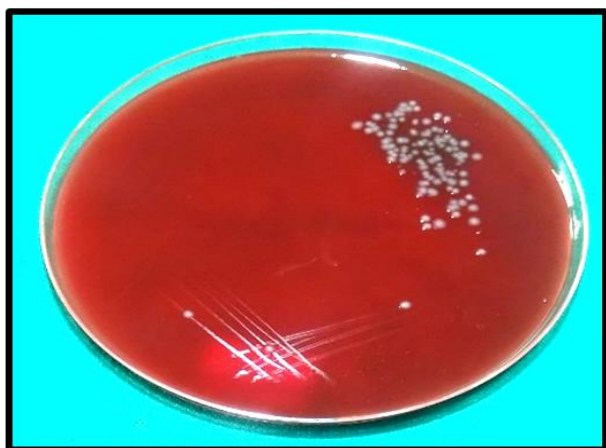


Fig 3: Group A – Culture plate showing *E faecalis* bacterial Colony Forming Units(CFU).

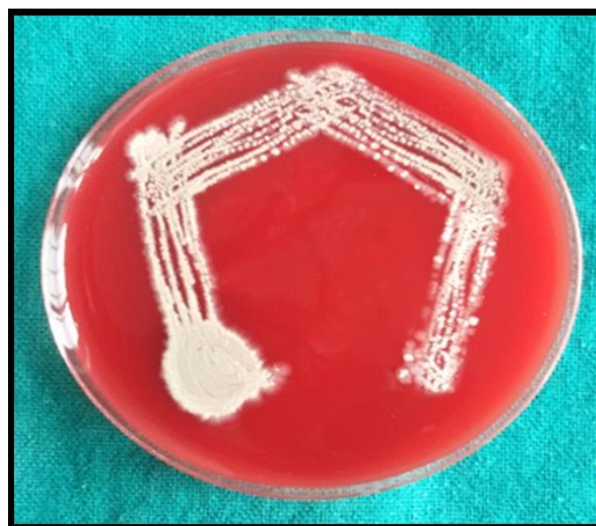
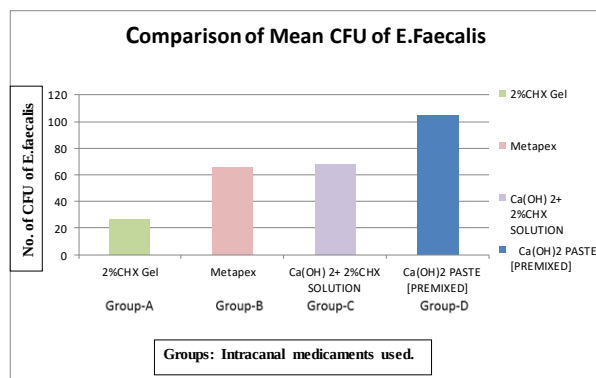


Fig 4: Group D–Culture plate showing *E faecalis* bacterial Colony Forming Units(CFU).



Graph I: Vertical Bar Graph.

DISCUSSION

Enterococcus faecalis is the most commonly implicated bacteria in asymptomatic persistent endodontic infections. The highly complex nature of this microorganism poses a great challenge to the clinician in its elimination. *E. faecalis* is known to colonize dentinal tubules, isthmus, apical ramifications, lateral and accessory canals of the root canal system and is the most persistent bacteria causing endodontic flare-ups or re-infection.³ The basis for successful endodontic treatment is the complete elimination of microorganisms from the root canal system. The use of a biocompatible intracanal medicament with potent antibacterial properties placed in the root canal space between the inter-appointments helps in completely eliminating the bacterial flora from the

Table 1: Analysis of Variance(One Way ANOVA) and Tukey's Post Hoc test.

Groups	N	E.Faecalis bacterial CFU. (Mean $\pm$ SD)		Significant Groups at 5% level	Non-significant Groups at 5% level
Group A	20	27.00 $\pm$ 1.38	P value = 0.0001HS	A vs B,C,D	
Group B	20	66.00 $\pm$ 3.38		B vs A,D	B vs C
Group C	20	68.00 $\pm$ 2.95		C vs A, D	C vs B
Group D	20	105.0 $\pm$ 1.92		D vs A,B,C	

HS: Highly significant, P: Probability, CFU: Colony Forming Units.

root canal system, thus significantly increasing the success of root canal treatment.^{3,4}

In the present study, Lentulo spiral(Mani, Inc, Prime Dental Products) engine driven instruments were used to place intracanal medicaments in the root canal space of all the teeth, as these instruments helps in the proper and uniform placement of the medicaments in the root canal space.^{9,10} In the present study, Medium-sized Canalbrush (Roeko canal brush, Coltene Whaledent) were used for the removal of intracanal medicaments from the root canal space, as they were more effective in the removal of intracanal medicaments even from narrower parts of the root canal with its better contact with the root canal surface.^{11,12}

In the present study, in Group A; 2% Chlorhexidine gel (Cavisept, Neelkanth Health Care, India) was used as intracanal medicament and it showed the highest antibacterial efficiency against E.faecalis compared to the other intracanal medicaments used in our study with the mean bacterial count of C.F.U as 27.00 and the same corroborates with a previous study of V ballal et al¹³ which also showed superior antibacterial efficiency of 2% Chlorhexidine gel as intracanal medicament against E. faecalis. Statistically significant difference was seen between Group A and other groups in our study.

In the present study, in Group B; Metapex [Meta Biomed Co. Ltd, Korea] was used as intracanal medicament. It contains 30% calcium hydroxide, 40.4% iodoform and 22.4% silicone oil. In our study, Metapex showed significant antibacterial efficiency against E. faecalis and it was

because of the synergistic effect⁵ of OH-ions & iodoform ions of metapex overcoming the buffering capacity of dentin and it is in accordance with the results obtained in a previous study of Ajitha et al⁵ which also showed significant antibacterial efficacy of metapex against E.faecalis. In the present study, it was also found that the antibacterial efficiency of metapex was lower compared to the 2%Chlorhexidine gel used and their was statistically significant difference in the antibacterial efficiency between group B and group A with the mean bacterial count of C.F.U in group B as 66.00. Punet Fulzele et al⁶ reported in his study that aqueous based preparations of calcium hydroxide should be chosen as intracanal medicaments rather than points or oil-based calcium hydroxide preparations and metapex has least antibacterial efficacy against E.faecalis compared to aqueous based preparations of calcium hydroxide.

In our study, in Group C; Calcium hydroxide powder (Safe plus, Neelkanth Health Care, India) was mixed with 2% Chlorhexidine gluconate solution (Asep-Rc, Anabond Stedman Pharma Research Pvt. Ltd, India) into a paste form and was used as intracanal medicament, it also showed good antibacterial efficiency against E.faecalis and this corroborates with the previous studies of Elka et al⁴ and Nageshwar rao et al,⁸ that 2% Chlorhexidine gluconate solution mixed with calcium hydroxide powder showed increased antibacterial activity against E.faecalis. However, the present study shows statistically significant difference in the antibacterial efficiency between group A, group C and group D with the mean bacterial count of C.F.U in group C as 68.00.

In the present study, in Group D; Calcium hydroxide paste in premixed form [Rc Cal Prime Dental Products. Pvt Ltd] was used as intracanal medicament. It is a water based medicament, acts by diffusion of hydroxyl ions through dentin and it showed least antibacterial effectiveness against *E. faecalis* compared to other intracanal medicaments used in the present study with the mean bacterial count of C.F.U as 105.0 and there was statistically significant difference in the antibacterial efficacy of group-D with that of all the other groups. It is in accordance with a previous study of Nidhi et al⁷ which also showed least antibacterial effectiveness of Calcium hydroxide paste on *E. faecalis* bacteria.

CONCLUSION

Within the limitation of this in-vitro study, it suggests that 2%Chlorhexidine gel used as an intracanal medicament has significantly higher antibacterial efficiency against *Enterococcus faecalis* and was more effective than Metapex, Calcium hydroxide powder mixed with 2% Chlorhexidine solution into a freshly made paste form and Calcium hydroxide paste in premixed form at 7 days interval. Further studies are required to prove the antibacterial efficiency of 2%Chlorhexidine gel in clinical situations. Calcium hydroxide paste in premixed form used as an intracanal medicament is found to be least effective with poor antibacterial efficiency against *E. faecalis*.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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