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RESEARCH ARTICLE

Comparative evaluation of 2% chlorhexidine , 0.2% cetrimide and 5% minocycline against *E.faecalis* : An *in vitro* study

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Abstract

Background: High prevalence of *E. faecalis* in root canal treated teeth with post-treatment disease suggests that this organism may be a key player in endodontic failure. Irrigants have a major role eradicating them from the complex root canal system.

Objectives: were to evaluate and to compare 2% chlorhexidine, 0.2% cetrimide and 5% minocycline by measuring the width of zone of inhibition of *E. Faecalis*

Methodology:

The antimicrobial activity of *E. faecalis* (ATCC 29212) was carried on sheep blood agar in nutrient broth. A total of 60 wells were made, 20 for each group and 50 µl of the test irrigant solution pipetted into each well. The test irrigants were 2% chlorhexidine, 0.2% cetrimide , 5% minocycline. The agar plates were carefully placed in CO₂ incubator at 37 °c for 48 hours. Zone of inhibition were measured using mm scale Statistical analysis was done using SPSS (version 17.0) software. ANOVA Test and Tukeys HSD tests were used to evaluate the mean and inter group comparison between the various groups. The p value was set at ≤0.05.

Results:

Among the experimental solutions 5% minocycline showed the largest zone of inhibition followed by chlorhexidine and cetrimide with statistical significant difference between all the groups, with p value <0.05.

Conclusion:

The present study concludes 5% minocycline is the best choice of irrigant when compared to 2% chlorhexidine and 0.2% cetrimide to eradicate *E. faecalis* in failed root canal cases.

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INTRODUCTION

Microorganisms are the main cause of pulpal infection, so the elimination of the microorganisms from the infected root canal system is the primary concern of the endodontic treatment. (Saeed Rahimi et al. , 2014). Disinfection is the paramount of successful endodontic treatment because the main cause of failure of pulp space therapy is the persistence of microorganisms and their reinfection. (Sundqvist G et al, 1998) The mechanical preparation alone is

not sufficient to disinfect the canal due to its complex structure which comprises of accessory canals, anastomoses and fins. (Rahimi S et al,2009) To increase the efficacy of mechanical preparation and bacterial removal, intracanal irrigants are used whose ideal properties include , its ability to dissolve pulp tissue remnants, remove the smear layer ,and be anti-microbial in nature.(Bystrom A et al, 1981) Different irrigants which are being used popularly in endodontics are 2.5% sodium hypochlorite,5% doxycycline, 0.2% chlorhexidine,0.2% cetrimide,5% minocycline.(Önçağ, Ö. Et al,2003)However, the efficacy of these procedures also depends upon the vulnerability of the various species. Gomes et al.(1996) found a link between anaerobic bacteria, especially black-pigmented Gram-negatives and the signs and symptoms of endodontic disease.(Zehnder M,2006) Facultative bacteria, such as *Enterococcus faecalis*, one of the most resistant species in the oral cavity and a possible cause of failure of root canal treatment, have also been isolated from pathologically involved root canals.(B. P. F. A. Gomes et al,2001) Hence the purpose of this study is to evaluate the efficacy of different irrigants such as 2% chlorhexidine , 0.2% cetrimide&5% minocycline against *E.faecalis*.

Objectives:

To evaluate and to compare 2% chlorhexidine, 0.2% cetrimide and 5% minocycline by measuring the width of zone of inhibition of *E. Faecalis*.

Material and methods:

The standard strain of *Enterococcus faecalis* (ATCC 29212) was used as a test strain. Four morphologically similar colonies were picked up from sheep blood agar medium with a wire loop and the growth was transferred to a test tube containing 4ml of sterile peptone water. The antimicrobial activity will be carried on sheep blood agar as per standard protocol of inoculum preparation in nutrient broth. After adjusting the inoculum to 0.5 McFarland tube the lawn culture was done on blood agar plates. A total of 3 wells per plate with a diameter of 6mm and depth 4mm were made equidistant from each other. A total of 60 wells were made, 20 for each group and 50 µl of the test irrigant solution pipetted into each well. The various test irrigants were studied are as follows;

Group A (CHX) – 2% chlorhexidine

Group B (CTR) – 0.2% cetrimide

Group C (MINO) – 5% minocycline

The agar plates were carefully placed in CO₂ incubator at 37 °c for 48 hours

Zone of inhibition were measured using mm scale passing through its diameter to the point of complete inhibition of growth on either side and the average was calculated using SPSS (version 17.0) software. ANOVA Test and Tukeys HSD tests were used to evaluate the mean and inter group comparison between the various groups. The p value was set at ≤0.05.

Results:

Among the experimental solutions 5% minocycline (group c) showed the largest zone of inhibition (mean) followed by chlorhexidine and cetrimide. (Table 1).Inter-group comparison shows 5% minocycline is the best among all the experimental groups with statistical significant difference with p value <0.05. (Table 2)

Discussion:

A successful endodontic therapy lies in the resolution of pulpal or periapical pathosis along with eradication of micro-organisms from the root canal. Researchers have noted that significant portions of root canal remain untouched even after biomechanical preparation.(Zahed mohammadi,2014) So, irrigants are necessary in addition to the mechanical eradication. The bacterial species *E.faecalis* was selected as the organism to be studied because studies have shown that previously treated failed root canal are 9 times more likely to be infected with *E.faecalis* than primary endodontic infections.(Alireza Adl et al,2014)*E.faecalis* is found to be the most frequent species in failed root canal cases because of its ability to tolerate the rough environmental changes which is believed to be due to its high alkali nature.(Mozayeni et al,2014) Zolleti et al evaluated that high prevalence of *E.faecalis* in root canal treated teeth with post-treatment disease, and suggested that this species may be a key player in endodontic failure.

To eradicate this highly virulent organism different irrigants have been tried. (REF) Results of the present study showed Minocycline among all the experimental irrigant was the best antimicrobial agent against *E. Faecalis*. Adl et al. showed that triantibiotic paste can effectively eliminate microorganisms along with sterilizing the root dentin. The results suggest that the triple antibiotic paste with either 2% chlorhexidine or normal saline would be the preferred medicament against *E. faecalis* and, among its three components; minocycline has the greatest antibacterial effect. Studies have also reported that microbes isolated from the root canal of filled teeth with

persistent periapical lesions were susceptible to tetracycline and doxycycline, which supports the trial of minocycline against *E.faecalis*. Tetracyclines penetrate bacterial cell by passive diffusion and inhibit bacterial growth by interfering with protein synthesis or by destroying cell membrane. Tetracyclines are distinguishable on the basis of mode of action : typical tetracycline such as minocycline are bacteriostatic while tetracycline derivatives are bactericidal. Minocycline is bacteriostatic in nature, this property may be advantageous because in the absence of bacterial cell lysis, antigenic byproducts such as endotoxin are not released. This could be the reason for the minocycline to show the best results with largest zone of inhibition in the present study.

Chlorhexidine (CHX) was not as good as Minocycline against *E. Faecalis*. CHX the most common irrigant used is a broad spectrum antimicrobial, with proven substantivity and capacity to inhibit the bacteria.(Hyun-Shik Kim,2013) It is a positively charged molecule which interacts with the negatively charged phosphate group on the microbial cell wall and causes its leakage to allow the chlorhexidine to penetrate the bacteria. Though widely used some studies even show additional use 0.2% chlorhexidine solution for canal irrigation can reduce the success of RCT. The results however showed the least efficacy with cetrimide, being a cationic surfactant with bactericidal activity it has the capacity to decrease the biofilm's mechanical stability.(Bailón-Sánchez ME,2014) So, cetrimide as an irrigant is used as surfactant with antimicrobial properties which can weaken the forces of biofilm and disrupt the extracellular polysaccharide matrix. Studies with the cetrimide have resulted in eradication of *E.faecalis*.(Arias-Moliz ,2010)Some studies have also showed 0.2% cetrimide showing higher substantivity than 0.2% chlorhexidine and substantivity similar to 2% chlorhexidine.(María Ferrer-Luque,2014)But the study conducted, proved that 2% chlorhexidine had better efficacy against *E.faecalis* than 0.2% cetrimide. While cetrimide and chlorhexidine had some antibacterial efficacy against *E.faecalis* but zones of inhibition they produced were smaller than that of minocycline which concurs with the previous literature.(Tamanna Alam.2005)Hence, it can be concluded that minocycline is most effective against eradicating *E.faecalis*, supporting minocycline as a potential intracanal irrigant in treatment of endodontic failures.

As *In vitro* study may not show full clinical potential of a tested material, as organic matter will interact with the endodontic disinfectants and the root canal is polymicrobial as well, so *in vivo* studies to be conducted to confirm the results of present study.

Conclusion:

Within the limitations present study concludes 5% minocycline is the best choice of irrigant when compared to 2% chlorhexidine and 0.2% cetrimide to eradicate *E.faecalis* in failed root canal cases.

Table 1: shows mean values and standard deviations of Zone of inhibition for various irrigants against *E.Faecalis*

	MEAN	STANDARD DEVIATION
MINOCYCLINE	23.7000	1.45458
CHLORHEXIDINE	16.7000	1.34164
CETRIMIDE	12.3000	1.21828

Table 1 shows mean values and standard deviations of Zone of inhibition for various irrigants against *E. Faecalis*

Table 2 : shows inter group comparison of zone of inhibition among the irrigants used.

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
minocycline	chx	7.00000*	.42426	.000	5.9790	8.0210
	cetrimide	11.40000*	.42426	.000	10.3790	12.4210
chx	minocycline	-7.00000*	.42426	.000	-8.0210	-5.9790
	cetrimide	4.40000*	.42426	.000	3.3790	5.4210
cetrimide	minocycline	-11.40000*	.42426	.000	-12.4210	-10.3790
	chx	-4.40000*	.42426	.000	-5.4210	-3.3790

*. The mean difference is significant at the 0.05 level.

Table 2 shows inter group comparison of zone of inhibition among the irrigants used.

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