ijcrr
Vol 04 issue 05
Category: Research
Received on:09/01/12
Revised on:23/01/12
Accepted on:03/02/12

ANTIMICROBIAL EFFICACY OF DIFFERENT ROOT CANAL PREPARATION TECHNIQUES USING K- FILES AND K- NITI FLEX – AN INVITRO STUDY

Arul Pari[1], Arun Prasad Rao[2], Thayumanavan[2], C.Vijay[3]

[1]Department of Pedodontics and Preventive Dentistry, Priyadarshini Dental College and hospitals, Chennai (Tamil Nadu)
[2]Department of Pedodontics and Preventive Dentistry, Rajah Muthiah Dental College and hospitals, Chidambaram (Tamil Nadu)
[3]Department of orthodontics, Priyadarshini Dental College and hospitals, Chennai (Tamil Nadu)

E-mail of Corresponding Author: arulpari@yahoo.co.in

ABSTRACT

Enterococcus faecalis is the most commonly isolated or detected species from oral infections including marginal periodontitis, infected root canal and periradicular diseases. So, the elimination of bacteria, their products and substrate enhances the success rate of endodontic therapy. Thus, this study was done to compare the antimicrobial efficacy of two different root canal techniques using K-file and K-Nitiflex. 30 intact right and left maxillary premolars extracted for orthodontic reasons were included in this study, after pulp extirpation, teeth were decoronated and autoclaved. Root canals were inoculated with *Enterococcus faecalis* suspension incubated at 37° for 24 hours. Six teeth were randomly allocated to five groups such as Step back preparation using K-file, Step back preparation using K-Nitiflex , Standard preparation using K-file, Standard preparation using K-Nitiflex and Saline irrigation. A pre-treatment and Post- treatment sample was obtained and its prevalence was evaluated using cultivation. All the data were statistically evaluated. The results suggest that the reduction in bacterial counts were statistically significant with step back using K-file and K-Nitiflex file and also standard technique using K-file and K-Nitiflex file. Finally, the present study concludes, that mechanical effects along with the adjunctive chemical substances possessing antimicrobial properties would effectively eradicate root canal infections.

Keywords: Root canal therapy, Enterococcus faecalis, step back technique, standard technique, K-Nitiflex, K-File.

INTRODUCTION

Root canal therapy is an invaluable measure to preserve teeth that would otherwise need to be extracted. With a better understanding of root canal anatomy and improved materials, advancing technology, root canal therapy is achieving an increasingly high over all success rates. However bacteria inside the root canal system have significant impact on this success rate.[10] A few bacterial species, predominantly facultative anaerobes[16] causing apical periodontitis are responsible for the root canal failures.[15,17]

Root canal failures result from these micro-organisms that have leaked into the canal after the obturation or from bacteria not eliminated during therapy. Therefore improving the cleaning and disinfection phase of treatment is of crucial importance and has led to the advancement of instrumentation and irrigation.[27]

During endodontic treatment, bacterial reduction or elimination may be achieved by both chemomechanical preparation and intracanal dressings. The removal of

irritants from the root canal is conducted by means of mechanical action of instruments with flow and backflow of the irrigant solution.[22] In addition; antibacterial irrigants may significantly help to eliminate bacterial cells from the root canal system.

Sundqvist et al (1998) recovered numerous species of anaerobic bacteria from failed root canal systems. Some of the bacterial species found out were Enterococcus faecalis, Streptococcus anginosus, Bacteriods gracilus and Fuso bacterium nucleatum.[26] From all the cases studied Enterococcus faecalis was found to be the most prevalent agent for the cause of failures.[26]

Enterococcus faecalis is a non spore forming fermentative, facultative anaerobic, gram positive coccus. Infact, the prevalence of Enterococci in primary endodontic infections and in persistent infections had been almost exclusively reported by using cultivation.[16,26]

Bystrom and Sundqvist (1985) used physiological saline solution during instrumentation; found that bacteria persisted in about half of the cases despite treatment on five successive occasions. Teeth where the infection persisted were those with a high number of bacteria in the initial sample.

Bystrom and Sundqvist (1985)[3] found *Enterococcus faecalis* to be highly resistant to antimicrobial medicaments, such a calcium hydroxide. Efforts to eliminate bacteria from the root canal system are accomplished by thorough cleaning and shaping of the root canal followed by an interim dressing of calcium hydroxide and adequate filling of the empty space.[3]

An adequate cleaning and enlargement of root canal is a prerequisite for a successful root filling. To deal with complex problem of preparing curved root canals, several instrumentation techniques such as step back method, standard method, balanced method and ultrasonic method were proposed.[19] Moreover, recent advances in technology allowed the introduction of endodontic files manufactured from nickel-titanium alloy, with more elastic flexibility, as well as improved resistance to torsional fracture.

AIMS AND OBJECTIVES

1. To determine the efficacy of step back technique using K-file and K-Nitiflex in reducing *Enterococcus faecalis* in artificially inoculated root canals.
2. To determine the efficacy of standard technique using K-file and K-Nitiflex in reducing *Enterococcus faecalis* in artificially inoculated root canals.
3. To compare the efficacy in the reduction of microbial counts among the step back and standard technique using K-file and K-Nitiflex.

MATERIALS AND METHODS

The present study was conducted in Division of Pedodontics and Preventive Dentistry in association with Department of Microbiology, RMDCH, Annamalai University.

Selection criteria:

- Thirty intact human right and left maxillary first premolars
- Teeth extracted for orthodontic reasons.
- Teeth that had two root canals.

Materials used:

- K-file
- K-Nitiflex
- Pfizer selective medium
- Inoculation loop
- Bacteriological incubator
- Petriplates
- Aluminium foil
- Airotor Handpiece
- Endoblock (Dentsply)
- Micropipette
- Paper points
- Autoclave, Hot air oven
- Vials

- Standard *Enterococcus faecalis* ATCC (259212)

Conventional access preparation were made, decoronated and autoclaved.

Inoculation of root canal

Enterococcus faecalis suspension equivalent to 0.5 McFarland's standard at the log phase of growth was used for inoculation. Teeth randomly were inoculated with O.lml/canal suspension under aseptic conditions. Even distribution along the working length was facilitated using sterile K # 15 file. The end point of the preparation length was assigned by macroscopic control at a distance 1mm coronal from the root apex. Thereafter teeth were wrapped in sterile aluminium foil and incubated at 37°C for 24 hr.

Six teeth were randomly allocated to each of five groups

Group 1: Step back preparation using K-file

Group 2: Step back preparation using K-Nitiflex

Group 3: Standard preparation using K-file

Group 4 : Standard preparation using K-Nitiflex

Group 5 : Saline irrigation

Sample collection:

Pre treatment sample was obtained by inserting ≠15 moistened sterile paper points into the root canal and removed after 30 seconds. The paper points were placed into 200µl sterile physiological saline and vortexed for 30 seconds.

In all the four instrumental groups (group 1- 4) the size of the master files was ≠ 40. After preparation, canals were irrigated with 0.1ml of sterile physiological. In group 5 treatment consisted' irrigation with 1ml of physiological saline only. Post-treatment samples were obtained from all 5 groups.

Pre and post treatment samples of each tooth were serially diluted in the physiological saline to give a final dilution of 10^1, 10^2, and 10^3.

Preparation of media

The media used in this study for culturing *Enterococcus faecalis* was Pfizer selective Enterococcus agar. It was prepared as per the manufacturer's protocol.

Agar Plating and Colony Counting

100µl of diluted samples were pipetted out on the surface of Pfizer selective Enterococcus agar and spread evenly. The plates were then incubated at 37° C for 24 hours. To avoid bias, procedures was carried by the same investigator. Colonies of ***Enterococcus faecalis*** were identified as a 0.5 mm entire edge, raised colonies with a brown halo.

The colony count of each plate was recorded and the mean CFU/ml was determined.

Statistical evaluation:

All the data was entered into a data based on Microsoft excel and analysed using SPSS with paired't' test and one way ANOVA. Difference at the 5% level ($P<0.05$) were considered statistically significant.

RESULTS

The average *Enterococcus faecalis* were found to be 2833333.3 with the standard deviation of 467618.08 before step back technique using K-file preparation (fig.3) and was reduced to 718416.67 (fig.4) after preparation whereas 1161666.7 with the SD of 638699.20 before step back technique using K-Nitiflex preparation (fig.5) and was reduced to 228500.00 (fig.6) after preparation.

The average *Enterococcus faecalis* was found to be 2075000.0 with the standard deviation of 2630480.9 before standard technique using K-file preparation (fig.7) and was reduced to 257333.33 (fig.8) after preparation whereas 1195000.0 with the SD of 389551.02 before standard technique

using K-Nitiflex preparation (fig.9) and was reduced to 72000.00 (fig.10) after preparation.

The corresponding logarithmic values have been taken out to standardize the values. Table.1 indicates the significant reduction in the microbial count after both the techniques using K-file and K-Nitiflex preparation.

The average *Enterococcus faecalis* was found to be 1646666.7 with the standard deviation of 2475994.1 before saline irrigation (fig.11) and was reduced to 266666.67 (fig.12) after irrigation. The corresponding logarithmic values have been taken out to standardize the value. Table.1 indicates that there is no significant reduction in the microbial count after saline irrigation (Control Group).

Table 2 shows the mean reduction between before and after intervention for the four treatment groups. Since no significant reduction was found in the saline group, it was not considered for further analysis. Further analysis suggests that the non-significant result infers that the mean reduction was equal for all the four preparation technique

DISCUSSION

In the past decades, several authors have provided evidences that bacterial infections play a decisive role in the course of pulpitis and periapical inflammations. Accordingly, for root canal therapy the reduction or eradication of the bacterial population seems to be a justified goal.[2] The principles of root canal preparation are to remove all organic debris and microorganisms and to shape the walls of the canal to facilitate cleaning and obturation of the entire root canal space. The current concept of root canal preparation is not cleaning and shaping but shaping and cleaning.[4] For the preparation of root canal, not a single technique or instrument is unequivocally accepted as being optimal.

The standard preparation technique is still popular, but it is subject to strong criticism because in case of curved or oval root canal this method is not suitable.[3] In order to overcome the problems of the curved root canals, the step back technique was devised by Mullaney 1979.[18] Even though it overcomes the procedural errors of the standardized techniques problem still exists in severely curved canals. A special filing technique, A file with non-cutting tip, more flexible instruments are used to overcome some of the problems of curved canal.

In the past files and reamers were manufactured from either carbon steel or stainless steel. In **1988 Walia et al** reported files made from nickel-titanium alloy.[28] Nitiflex instruments may be as aggressive in removing dentin. In addition they are more resistant to wear than their stainless steel counter parts. They were more efficient than square shaped K-files in preparing curved root canal with circumferential filing techniques.[8] **Horland et al.** stated that stainless steel instruments reached greater penetration depth and cutting efficiency than nickel titanium instruments[6] Factors that may contribute to a persistent periradicular infection after treatment include intraradicular infection, extraradicular infection, foreign body reaction and cysts containing cholesterol crystals.[7]

The major cause of failure is the survival of microorganisms in the apical portion of the root filled tooth.[1] Unlike primary endodontic infections which are polymicrobial in nature, the microorganisms involved in secondary infections are composed of one or a few bacterial species.[12,25]

Enterococcus faecalis is a persistent organism that, despite making up a small portion of flora in untreated canals, plays a major role in the etiology of periradicular lesions after root canal treatment. It is a

normal commensal adapted to ecologically complex environments of the oral cavity, gastrointestinal and vaginal tracts.[14] It is often involved in persistent endodontic infections and it is one of the most resistant species found in the oral cavity.[24]

Enterococcus faecalis has widespread genetic polymorphisms.[21] It possess serine protease, gelatinase and collagen binding protein (Ace), which help it bind to dentin.[13] It is small enough to proficiently invade and live within dentinal tubules. It has the capacity to endure prolonged periods of saturation until an adequate nutritional supply becomes available.[9]

Enterococcus faecalis can gain entry into the root canal system during, between or at end of the treatment.[20] Therefore, it is important to consider regimens aimed at eliminating or preventing the infection of *Enterococcus faecalis* during each of these phases.

In the present study, K-files showed marked reduction in both techniques correlating with study done by **Dalton et al. (1997).**[5] When both technique were compared using K-file, step back showed better bacterial reduction. When above technique was compared using K-Nitiflex standard showed better bacterial reduction correlating with study done by Sequiera **et al.** (1999).[24] Thus, the reduction in the bacterial count was statistically significant, with both techniques using K-files and K-Nitiflex (Fig 1) correlating with study done by **Siqueira et al** (1999).[24]

No antibacterial irrigant was used. Elimination of bacteria was dependent on the mechanical action and irrigation with physiological saline which yielded a slight reduction in the bacteria.

It was also found that there was no statistically significant difference in the mean reduction in the bacterial count between the both techniques using K-file and K-Nitiflex (Fig 2) because we used maxillary premolars and the occurrence of severely curved roots is relatively rare in this group of teeth. This result is in line with those published by **Pataky (2002).**[19]

Although a considerable bacterial reduction was achieved by the techniques tested, bacteria were never thoroughly eliminated regardless of the instrumentation techniques and files used. Whereas minor anatomical irregularities may be incorporated in the preparation, such as fins and ramifications which possibly were not detected by radiographs might have restored bacteria. These areas are commonly unaffected by instruments and irrigation with physiological saline during canal preparation.

Therefore, the need to use antibacterial irrigants and medicaments to maximize the bacterial elimination from the root canal becomes necessary.

SUMMARY AND CONCLUSION

From the present study it was concluded that the reduction in bacterial counts were statistically significant with step back using K-file and K-Nitiflex file and also standard technique using K-file and K-Nitiflex file.

There was no statistically significant difference while comparing the mean reduction in the bacterial count between the standard and step back technique using K-file and K-Nitiflex.

The results indicated that the mechanical effects caused significant decrease in bacterial cell number in the root canal. But the mechanical means are insufficient to completely eradicate root canal infection and the use of adjunct chemical substances possessing antibacterial properties becomes necessary.

ACKNOWLEDGEMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to

authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. Baumgartner JC, Falker WA. Bacteria in the apical 5mm of infected root canals. / *Endod.* 1991:17: 380-3.
2. Bergholtz G, Crawford JJ. Endodontic microbiology in: Walton RE, Torbinejad Meds, Principles and practice of Endodontics. *Philadelphia* : WB Saunders. 1989: 277.
3. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochloride and EDTA in 60 cases of endodontic therapy. *Int Endod J.* 1985; 18: 35-40.
4. Carrotte P. Preparing the root canal. *British Dental Journal* 2004; 197: 603-613.
5. Dalton C, Orstavik D, Trope M. Intracanal bacterial reduction with nickel-titanium rotary instrumentation. / *Endod.* 1997; 23: 2.
6. Edgar Schafer, Klaus Bossmann, Antimicrobial efficacy of chlorhexidine and two calcium hydroxide formulation against *Enterococcus faecalis* invitro. / *Endod.* 2005; 31: 53-56.
7. Evans M, Davies JK, Sundqvist G, Figdor D, Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod j.* 2002;35:221-8.
8. Fadia E, Bou Dagher, and Ghassan M, Yared, Comparison of three files to prepare curved Root canals. / *Endod.* 1995; 21: 264-265.
9. Figdor D, Davies JK, Sundqvist G. Starvation Survival growth and recovery of *Entercoccus faecalis* in human serum. *Oral Microbiol Immunol.* 2003; 18: 234-9.
10. Friedman S, Mor C. The success of endodontic therapy - healing and functionality, *CD A Journal.* 2004; 32: 493-503.
11. Friedman S. Abitbols, Lawrence HP, Treatment outcome in endodontics. The Toronoto Study Phase I : Initial Treatment. / *Endod.* 2003; 29: 787-93.
12. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North an population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2000; 91: 579-86.
13. Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of *Enterococcus faecalis* proteases and the collagen-binding protein, pace, on adhesion to dentin. *Oral Microbial Immunol.* 2003; 18:121-6.
14. Jett BD, Huycke MM, Gilmore MS, Virulence of Enterococci. Clin Microbiol Rev. 1994; 7; 462-78.
15. Kakehashi S, Stanley HR. Fitzgerald RJ. The effect of surgical exposures of dental pulps in germ-tree and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1965; 20: 340-9.
16. Molander A, Reitc, Dahlen G, Kvist T., Microbiological status of root filled teeth with apical periodontitis. *Int Endod J* 1988: 31-1-7.
17. Moller A J, Fabricius R, Dahlen G, Ohman AE, Influence on periapical tissues of indigenous oral bacterial and necrotic pulp tissue in monkeys. *Scand J Dent Res.* 1981; 89: 475-84.
18. Mullaney TP. Instrumentation of finely curved canals. *Dent Clin North Am.* 1979; 23: 575-592.
19. Pataky, Ivan Nangi, Ayne Grigar, Arpad Fazekas. Antimicrobial efficacy of various root canal preparation techniques. / *Endod.* 2002; 28: 603-605.

20. Rocas IN, Siqueria JF, Santos KRN, Association of Enterococcus faecalis with different forms of periradicular diseases. / *Endod*. 2004; 30: 315-20.
21. Sedgley CM, Lennan SL, Lewell DB. Prevalence, Phenotype and genotype of oral Enterococci. *Oral Microbial Immunol* 2004; 19: 95-1001.
22. Siqueira JF Jr, Araujo MCP, Garcia PF, Fraga RL, Sobia Dantas CJ, Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. / *Endod.* 1997; 23: 499-502.
23. Siqueira Jr JF, Aetiology of root canal treatment failure : Why well treated canal fail *Int Endod J.* 2001; 34; 1-10.
24. Siqueira Jr. Kenioc Lima Fernades AC. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. / *Endod.* 1999; 25: 352-35.
25. Siren EK, Haapasalo MRP, Rantak, Salmi p, Kerosuo ENJ, Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. *Int EndodJ*. 1997;30:91-5.
26. Sundqvist G, Figdor P, Persson S, Sjogren, Microbiological analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998; 85: 86-93.
27. Torabinejad M, Khademi A, Babagoli J, Choy Johnsaw, A new solution for the removal of smear layer. / *Endod*, 2003; 29; 170-75.
28. Walia H, Brentley W, Gerstein H. An Initial investigation of the *t* bending and torsional properties of Nitinol root canal files. / *Endod*. 1988; 14: 346-50.

Table -1: **Mean and Standard Deviation of *Enterococcus faecalis* Counts before and after by different Root Canal Preparation Groups**

Group	Pre		Post		Difference	Paired 't'	P Value
	Mean	SD	**Mean**	SD			
Step Back K-file	2833333.3	467618.08	718416.47	924482.04	21,14,917	2.833	0.036
Step Back K - Nitiflex	1161666.7	638699.20	228500.00	322604.25	9,33,166.7	5.244	0.003
Standard K-file	2075000.0	2630480.9	257333.33	200844.88	18,17,667	2.822	0.037
Standard K-Nitiflex	1195000.0	389551.02	72000.000	50785.825	11,23,000	9.198	0.000
Saline Group (Control Group)	1646666.7	2475994.1	266666.67 >	46761.808	13,80,000 *	3.490	0.17

SD - Standard deviation
Significant at $P < 0.05$.

Table -2: **Comparison of mean reduction in the microbial *(Enterococcus Faecalis)* counts between the groups**

Sl.No	**Difference**		**One Way ANOVA 'F'**	**P Value**
	Mean	**Standard Deviation**		
Step Back K-File	2114917	889543.16	0.539	0.661
Step Back K - Nitiflex	933166.7	538765.40		
Standard K-file	1817667	2667538.31		
Standard K-Nitiflex	1123000	405809.31		

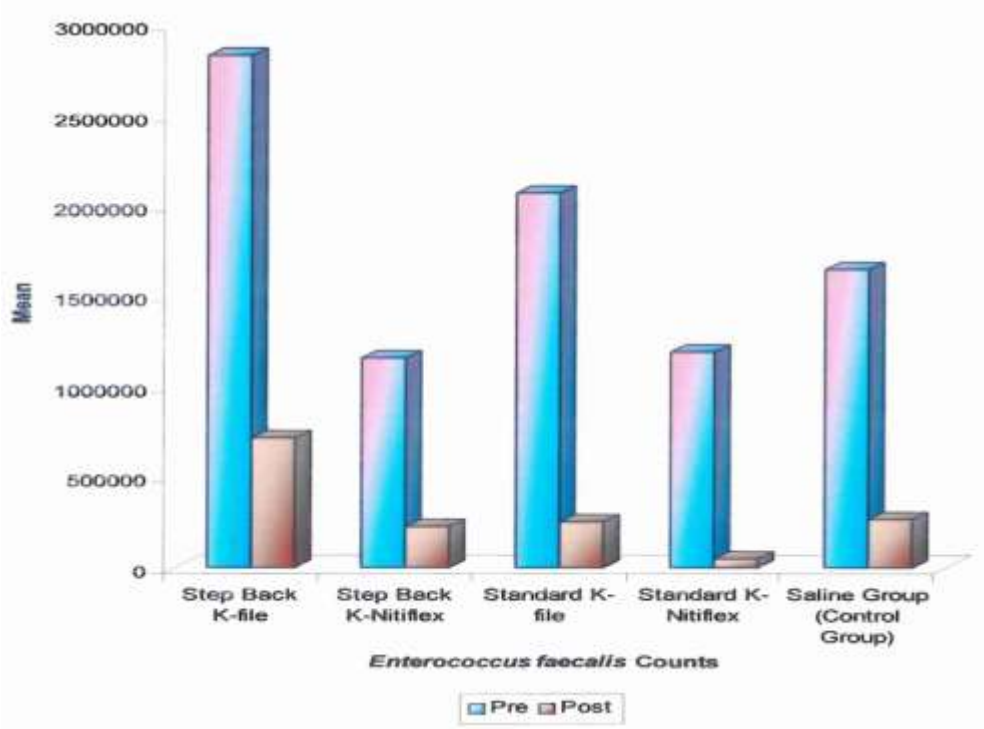

Figure.1

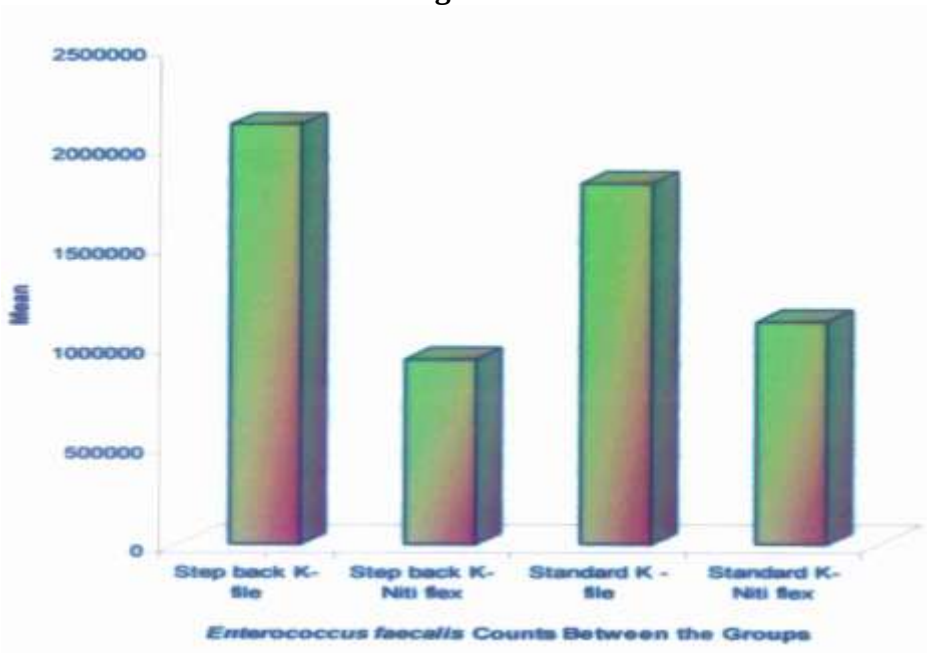

Figure.2

Figure.3

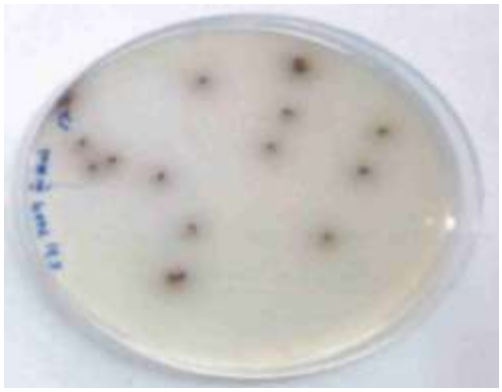

Figure.4

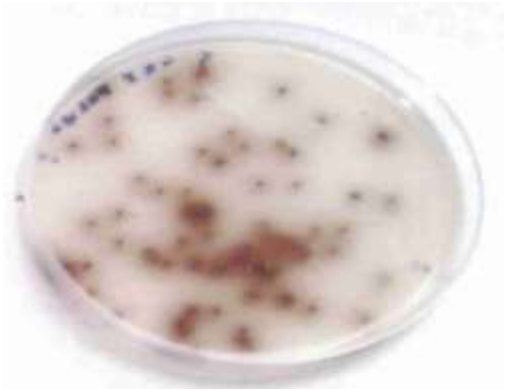

Figure.5

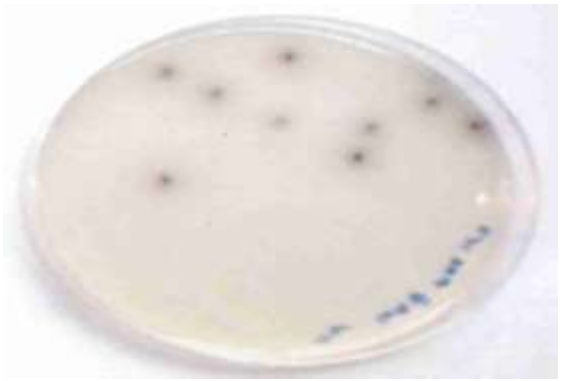

Figure.6

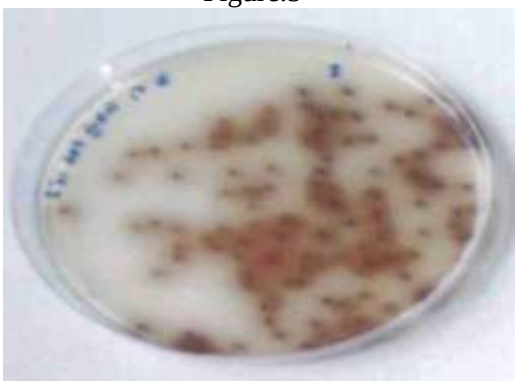

Figure.7

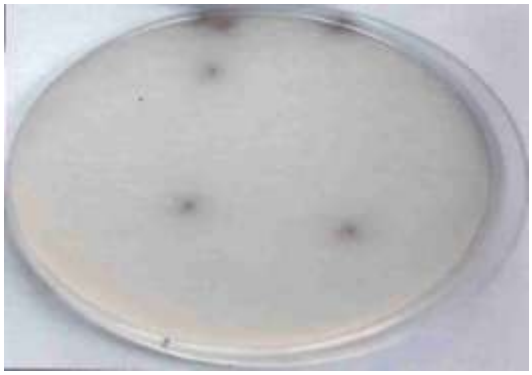

Figure.8

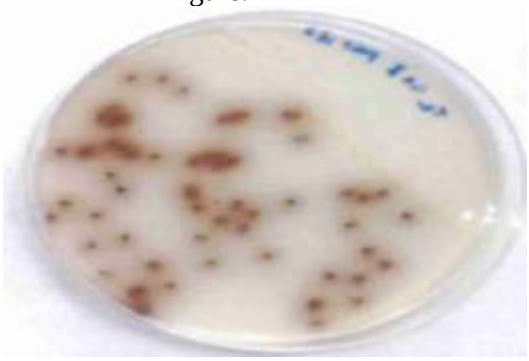

Figure.9

Figure.10

Figure.11

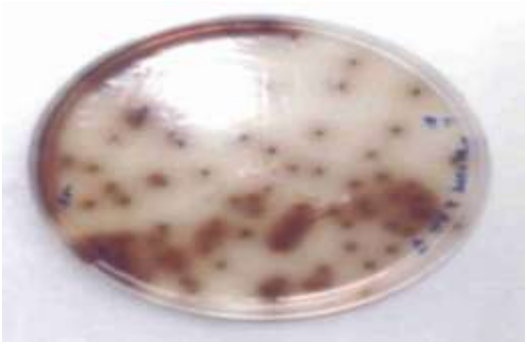

Figure.12

Legends

Figure.1 Bar Diagram showing the *Enterococcus faecalis* Counts before and after by different Root Canal Preparation Groups

Figure.2 Comparison of mean reduction in the microbial *(Enterococcus faecalis)* counts between the groups

Figure.3 *Enterococcus faecalis* colonies before step back preparation using using K-file.

Figure.4 *Enterococcus faecalis* colonies after step back preparation using using K-file.

Figure.5 *Enterococcus faecalis* colonies before step back preparation using using K-Nitiflex

Figure.6 *Enterococcus faecalis* colonies after step back preparation using using K-Nitiflex.

Figure.7 Enterococcus *faecalis* colonies before standard preparation using using K-file.

Figure.8 *Enterococcus faecalis* colonies after standard preparation using using K-file.

Figure.9 *Enterococcus faecalis* colonies before standard preparation using using K-Nitiflex.

Figure.10 *Enterococcus faecalis* colonies after standard preparation using using K-Nitiflex.

Figure.11 *Enterococcus faecalis* colonies before saline irrigation.

Figure.12 *Enterococcus faecalis* colonies after saline irrigation.