

## A Comparison Of The Antibacterial Efficiency Of MTAD (Mixture Of Tetracycline, Citric Acid And Detergent), 2.5% Sodium Hypochlorite And 2% Chlorhexidine Root Canal Irrigants Against Enterococcus Faecalis In Root Canals Of Single Rooted Mandibular Premolars- An In Vitro Study

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**Abstract: Introduction:** Historically, countless compounds in aqueous solution have been suggested as root canal irrigants. MTAD shows promise as an endodontic irrigant but there are very few studies comparing its antimicrobial effectiveness with NaOCl and Chlorhexidine.

**Objectives:** To compare the antibacterial efficiency of MTAD, 2.5% Sodium Hypochlorite and 2% Chlorhexidine root canal irrigants against Enterococcus faecalis in root canals of single rooted mandibular premolars.

**Method:** Inoculum of 10 µl, 24hrs pure culture Enterococcus faecalis was suspended into root canals of forty extracted, decoronated, double sterilized, human mandibular single rooted premolar teeth and incubated for 48hrs at 37°C. Teeth were randomly divided into 4 groups of 10 teeth in each group. The teeth were instrumented in step back technique till size 40 K- file and irrigated with respective root canal irrigants as per the groups. In Group A- MTAD, Group B- 2.5% NaOCl, Group C- 2% Chlorhexidine and Group D- normal saline was used. Bacterial sample from each tooth was collected and bacterial culture was carried out using standard Loop technique. Inoculated plates were incubated at 37°C for 48hrs. Bacterial colonies were counted using colony counter.

**Statistically Analysed:** Using One way ANOVA and LSD Post Hoc test.

**Results:** There was a statistically significant difference in the antibacterial efficiency between all the groups.

**Conclusion:** Within limitations of this study it can be inferred that MTAD has best antibacterial efficiency against Enterococcus faecalis when compared to 2.5% Sodium Hypochlorite and 2% Chlorhexidine.

**Keywords:** Chlorhexidine; Enterococcus faecalis; Irrigants; MTAD; Sodium Hypochlorite.

### I. Introduction:

Although chemo-mechanical preparation of the root canal system is able to reduce the number of bacteria, complete canal disinfection is difficult because of the internal complexity of root canal systems [1,2]. Thus root canal irrigation forms an integral part of the endodontic therapy to ensure bacterial elimination [3,4] and for the digestion of the organic tissue remnants as it allows for cleaning beyond what might be achieved by root canal instrumentation alone [5,6].

According to Zehnder (2006)[7] root canal irrigants ideally should:

- Have a broad antimicrobial spectrum and high efficacy against anaerobic and facultative microorganisms organized in biofilms
- Dissolve necrotic pulp tissue remnants
- Inactivate endotoxin
- Prevent the formation of a smear layer during instrumentation or dissolve the smear layer once it has formed

Furthermore, as endodontic irrigants come in contact with vital tissues, they should be systemically nontoxic, non-caustic to periodontal tissues and have little potential to cause an anaphylactic reaction.

Historically, countless compounds in aqueous solution have been suggested as root canal irrigants. Prior to 1940's Water was the most commonly used irrigant. During 1940's proteolytic enzymes like streptokinase,

streptodomas, papain, enzymal etc. were used as irrigating solutions because of their tissue dissolving property. In 1943, Grossman introduced the concept of using oxidizing agents (hydrogen peroxide and Gly-Oxide) as irrigants. In 1945, Daniel formulated an irrigating solution comprising of aminoacridine. In 1970's chelating agents (EDTA) were used increasingly because of their biologically acceptable properties. Other irrigants include acids (citric and phosphoric), alkaline solutions (sodium hypochlorite, sodium hydroxide, urea and potassium hydroxide), local anaesthetic solutions and normal saline [8].

Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker (1936)[9]. It has been recommended as an irrigant solution in the treatment of infected root canals because of its well-known bactericidal action [10, 11, 12]. The major disadvantages of NaOCl are: (a) significant toxicity when injected into peri-radicular tissues (b) disagreeable smell and taste (c) risk of bleaching clothes (d) corrosion of metal objects [13] (e) inability to remove smear layer when used alone [14,15]. The biocompatibility problems associated with the use of concentrated NaOCl have led to the use of substances with known antimicrobial properties and less toxicity, such as chlorhexidine.

Chlorhexidine is bacteriostatic at low concentrations of 0.2%, bactericidal at high concentrations of 2% , adsorbs to dental tissue and mucous membrane, resulting in its prolonged gradual release at therapeutic levels [16] and is effective in eliminating bacteria penetrate up to 500 µm within dentinal tubules [17]. Despite its usefulness as a antimicrobial irrigant with low toxicity, Chlorhexidine cannot be advocated as the main irrigant in standard endodontic cases, because: (a) Chlorhexidine is unable to dissolve necrotic tissue remnants [18] (b) Chlorhexidine is less effective on Gram-negative than on Gram-positive bacteria [19, 20].

A new irrigating solution, MTAD, containing a mixture of a tetracycline isomer, an acid and a detergent, has demonstrated its ability to remove the smear layer, disinfect contaminated root canals and eradicate *E. faecalis* [21, 22, 23]. There is no doubt MTAD has good antimicrobial properties but there are very few studies comparing its antimicrobial effectiveness with NaOCl and Chlorhexidine. It is by no means clear if it is better than NaOCl and Chlorhexidine as an endodontic irrigant.

The intent of the current study was to compare the antibacterial efficiency of MTAD (Mixture of Tetracycline, Citric Acid and Detergent), 2.5% Sodium Hypochlorite and 2% Chlorhexidine root canal irrigants against *Enterococcus faecalis* in root canals of single rooted mandibular premolars. Our design allowed direct comparison of each irrigant against *Enterococcus faecalis*.

## II. Materials and methods

The study was carried in the Post graduate section of Department of Conservative Dentistry and Endodontics, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India. Forty extracted human mandibular single rooted premolar teeth (n = 40), non carious with matured, closed apices were collected from the oral surgery Department of Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India. Each tooth was radiographed to confirm for the presence of a single canal. All the teeth were decoronated with a diamond disc using a low speed straight hand piece (NSK EX-6B, Nakanishi Inc. Japan), so as to standardize roots of all the teeth (approximately 14mm) to the same length. Working lengths for all the teeth were determined using a size 15 K-files (Dentsply, Germany), measured with help of Mini Endo-block (endogauge)( Maillefer, Dentsply Switzerland) and radiographs were taken and recorded. Then all the teeth were double sterilized in an autoclave at a temperature of 121°C under 15 lbs of pressure for 20 minutes. The bacterial strain used in the present study was *E. faecalis* (ATCC 29212). It was grown in Brain Heart Infusion (BHI) (Himedia Laboratories. Pvt. Ltd, Mumbai, India) broth for 24 hrs at 37°C. An inoculum of 10 µl, 24hrs pure culture suspension of *E. faecalis* was suspended into root canals of all the teeth by using a tuberculin syringe (Romsons juniors India unit II Agra, India) inside the laminar air flow under fully aseptic conditions. Later each tooth was transferred carefully into a test tube containing 4ml of sterile normal saline and incubated for 48hrs at temperature of 37°C. After incubation, the teeth were removed from the test tubes and were rinsed thoroughly with sterile normal saline. All the teeth were then randomly divided into four test groups (n = 10 each) and each group was irrigated with different irrigants.

**Group A:** MTAD (Dentsply Tulsa Dental, Dentsply international, Inc. Tulsa, United States) was used as irrigant

**Group B:** 2.5% Sodium hypochlorite (Prevest Denpro Limited Jammu, India) was used as irrigant. 2.5 % Sodium hypochlorite was prepared from 5 % Sodium hypochlorite by adding distilled water to it in ratio 1:1.

**Group C:** 2% Chlorhexidine (Septodent Healthcare India Pvt. Ltd. Maharashtra, India) was used as irrigant

**Group D:** Normal saline (Nirma Limited Gujarat, India) was used as irrigant

**Group A:** Here only MTAD was used as root canal irrigant.

As per the working lengths determined, root canals of all the teeth were instrumented with step back technique and the canals were enlarged using K-file upto size 40 no. K-file. During the process of instrumentation, canals were irrigated with 2ml of MTAD per tooth. Following completion of instrumentation, a final rinse of each root

canal was carried out by using 5ml of MTAD for 5 minutes. After accomplishing the removal of excess moisture from the canal with help of size 35 no. absorbent paper point (Maillefer, Dentsply, China) , a dry sterile standardized absorbent paper point of size 40 was kept in the root canal to the full working length for 10 seconds. Later the paper point was taken out and transferred into a test tube containing 2ml sterile normal saline and was vortexed for 1 min. Then from each test tube sample, bacterial culture was carried out using a standard Loop technique for semiquantitative culture on 5% sheep blood agar and the inoculated plates were incubated at 37°C for 48hrs under aerobic conditions. This entire procedure was carried out in complete aseptic conditions. Finally with help of colony counter bacterial colonies were counted.

**Group B:** The entire procedure was carried out just similar to that of Group A, except that instead of MTAD, 2.5% NaOCl was used as root canal irrigant.

**Group C:** The same procedure was carried out here also as described for Group A, except that irrigation was carried out using 2 % Chlorhexidine.

**Group D:** The same procedure was carried out as described for Group A, except that sterile normal saline was used as an irrigant.

Counting of the Colony forming units (C.F.U) per plate for all groups was done. The results were statistically analysed.

### III. Observations and Results

Counting of the Colony forming units (C.F.U) per plate for all groups was done. The results were statistically analysed. Group A had colony forming units (C.F.U) in the range 5-16 while the group B showed 17-61 range of colony forming units. Group C had 62-130 whereas group D showed highest range of 72-296 colony forming units.

One way ANOVA analysis among the four groups is shown in Table 1.

**Table 1: One Way Anova Analysis**

Group	Range	Mean ± SD	One-Way ANOVA
A	5 – 16	9.30 ± 3.06	F = 20.985 p < 0.001**
B	17 – 61	44.80 ± 13.18	
C	62 – 130	81.60 ± 22.46	
D	72 – 296	138.30 ± 71.42	

F= F-ratio

\* p < 0.01; Significant

\*\* p < 0.001; Highly significant

In Table 1 we see an F-ratio (F) of 20.985 (p < 0.001). This p (F) tells that there is highly significant difference among the four groups of root canal irrigants. Overall group comparisons of p value was done using Post-hoc LSD test as shown in Table 2.

**Table 2: Overall Group Comparisons of p Value**

Group Comparisons	p value (Using Post-hoc LSD)
A vs. B	0.044*
A vs. C	<0.001**
A vs. D	<0.001**
B vs. C	0.037*
B vs. D	<0.001**
C vs. D	0.002*

\* p < 0.01; Significant

\*\* p < 0.001; Highly significant

Thus if the groups are overall compared with each other (Fig. 1&2) for antibacterial activity, it can be concluded that group A (MTAD alone was used as an irrigant), shows the maximum efficiency against Enterococcus faecalis, followed by group B (only 2.5% NaOCl was used as an irrigant) and the least effective is the control group D where only saline was used as an irrigant.

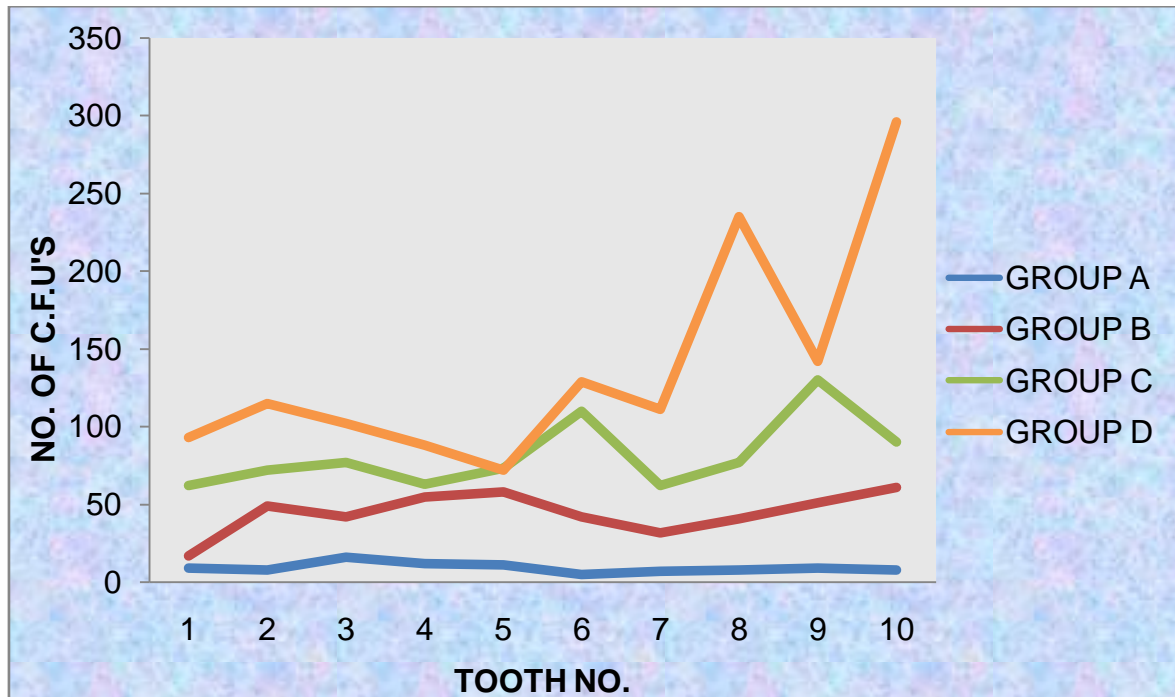


Figure 1: comparison of number of C.F.U'S among all groups

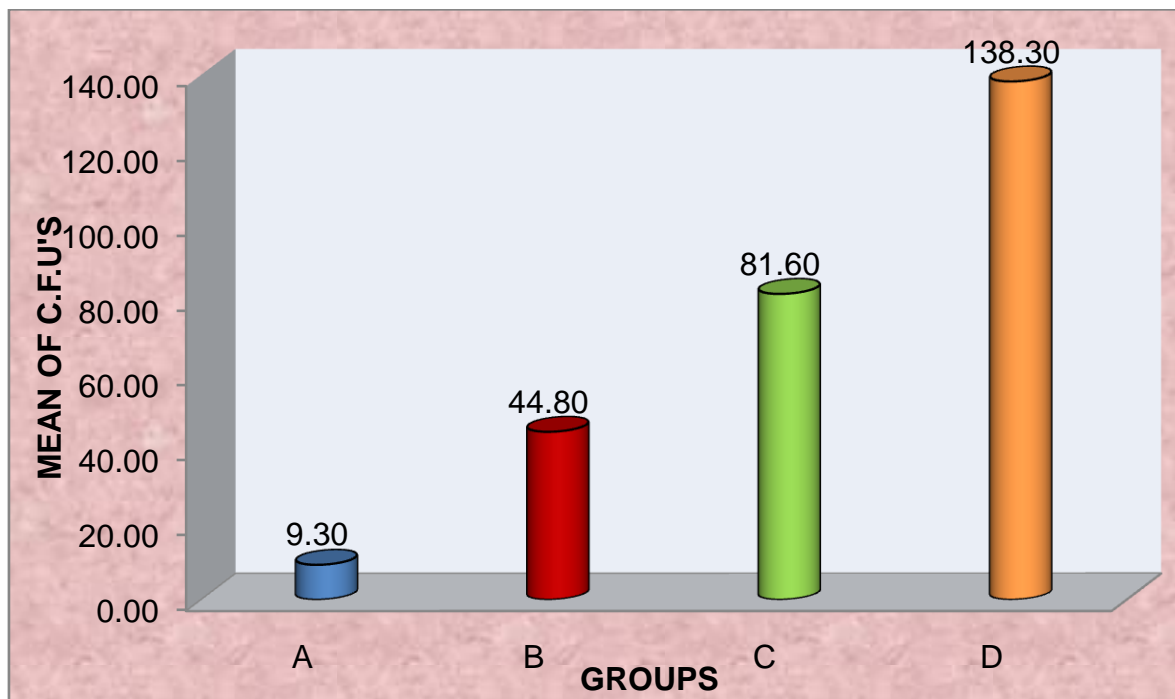


Figure 2: mean of C.F.U'S in all groups

#### IV. Discussion:

*E. faecalis* was chosen as the primary test organism because it is the most resistant bacteria found in the root canal system and has been associated with treated root canal failures. Its prevalence in such infections ranges from 24-77 % [24]. It was also chosen because recent studies claimed that MTAD is an effective final irrigating solution for eradication of *E. faecalis* [22,25].

2.5 % concentration of NaOCl was used because NaOCl is highly toxic to vital tissues at undiluted high concentrations. At higher concentration, for example, 1:10 (vol/vol) dilution, the tissue irritation may be substantial [26] and displacing highly concentrated NaOCl into periapical tissues can cause severe tissue damage [27]. Also there is some dispute regarding the most effective concentration of NaOCl, although

theoretically the concentration should be kept to the lowest level that is effective. When used in contaminated canals of extracted teeth, Siqueira *et al.* (2000)[28] found no difference in the antibacterial effect of 1%, 2.5% and 5.2% NaOCl. They suggested that copious irrigation with NaOCl may maintain a chlorine reserve that is sufficient to eliminate bacterial cells and compensate for the effect of concentration. Berber *et al.* (2006) [29] found no differences among various concentrations of NaOCl in reducing *E. faecalis* within root canals and dentinal tubules but only at the higher concentrations was NaOCl able to disinfect the dentinal tubules independent of the canal preparation technique used. Others also demonstrated that lower concentrations are as effective or ineffective in eliminating bacteria [30]. Since the proteolytic effect of NaOCl is dependent on the amount of free available chlorine that is used up during the process by reacting with inorganic reducing substances, frequent irrigation with a lower concentration may achieve as much of a proteolytic effect as the use of a higher concentration [28]. In the light of these clinical studies, it can be concluded that the NaOCl has an efficient antimicrobial effect at low concentration and it shows proportional increase in toxicity with higher concentration with no demonstrable clinical effectiveness.

2% concentration of Chlorhexidine was chosen for study because chlorhexidine is bacteriostatic at low concentrations of 0.2%. At high concentrations of 2% chlorhexidine is bactericidal and adsorbs to dental tissue and mucous membrane, resulting in its prolonged gradual release at therapeutic levels [16]. Aqueous solutions of 0.1 to 0.2% are recommended for chemical plaque control in the oral cavity, while 2% is the concentration of root canal irrigating solutions usually found in the endodontic literature [31].

In this study only MTAD (i.e. without initial use of 1.3% NaOCl) was compared for its antimicrobial efficiency because studies have shown that the cleanest canals were obtained if NaOCl was used before a final soak and flush with MTAD [25]. The studies comparing the antimicrobial effect of MTAD alone (i.e. without initial use of 1.3% NaOCl) with other irrigants are lacking.

The results of present study show that for the group comparison of A vs. B, the p value is 0.044 which is significant. Thus irrigant of group A i.e. MTAD has better antibacterial activity than irrigant of group B i.e. 2.5 % Sodium Hypochlorite. Similarly p value for comparison of group A vs. C and group A vs. D is <0.001 which is highly significant indicating that antibacterial efficacy of MTAD is much better than 2% Chlorhexidine and normal saline respectively.

p value for group comparison of B vs. C is 0.037 which is significant. Thus irrigant of group B i.e. 2.5 % Sodium Hypochlorite has better antibacterial efficacy than irrigant of group C i.e. 2 % Chlorhexidine. For the group comparison of B vs. D, the p value is <0.001 which is highly significant indicating that antibacterial efficacy of 2.5 % Sodium Hypochlorite is much better than Normal saline.

The results show that for the group comparison of C vs. D, the p value is 0.002 which is significant. Thus irrigant of group C i.e. 2 % Chlorhexidine has better antibacterial activity than irrigant of group D i.e. Normal saline.

Thus if the groups are overall compared with each other for antibacterial activity, it can be concluded that group A (MTAD alone was used as an irrigant), shows the maximum efficiency against *Enterococcus faecalis*, followed by group B (only 2.5% NaOCl was used as an irrigant) and the least effective is the control group D where only saline was used as an irrigant.

Torabinejad *et al.* (2003)[25] found MTAD was effective in killing *E. faecalis* up to 200 x dilution whereas NaOCl ceased to exert its antibacterial activity beyond 32 x dilution. Shabahang & Torabinejad (2003) [21] showed that the combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse was significantly more effective against *E. faecalis* than the other regimens. Newberry *et al.* (2007) [32] showed that MTAD in conjunction with 1.3% NaOCl was effective in completely eliminating growth in 7 of 8 strains of *E. faecalis*. Similar to these results, Davis *et al.* (2007)[33] found that MTAD showed significantly more zones of microbial inhibition for *E. Faecalis* than NaOCl, Chlorhexidine and Dermacyn (a superoxidized water).

The results of the our study done agree with these in-vitro studies and indicate that MTAD has better antibacterial activity than 2.5% sodium hypochlorite and 2 % Chlorhexidine.

However there are certain studies that display less than optimal antimicrobial activity of MTAD. Kho & Baumgartner (2006)[34] demonstrated no difference in antimicrobial efficacy for irrigation with 5.25% NaOCl/15% EDTA versus irrigation with 1.3% NaOCl/MTAD in the apical 5 mm of roots infected with *E. faecalis*. Dunavant *et al.* (2006) [35]found that 6% and 1% NaOCl were significantly more efficient in eliminating *E. faecalis* biofilms than other irrigants such as 2% CHX, MTAD and commercial preparations of EDTA. A study by Baumgartner *et al.* (2007)[36] found no growth of *E. faecalis* in root canals irrigated with 5.25% NaOCl/ 15% EDTA, whereas 50% of the canals irrigated with 1.3% NaOCl/MTAD demonstrated growth of *E. faecalis*. Less than optimal antibacterial effect of MTAD in these studies could be because of the difference in simulated clinical conditions, methodology, microbial sampling procedures or because of difference in the study model.

## V. Conclusion

Within the limitations of this study the following conclusions can be inferred that MTAD has best antibacterial efficiency against *Enterococcus faecalis* when compared to 2.5% Sodium Hypochlorite and 2% Chlorhexidine. Further studies are required to prove the efficiency of MTAD in clinical situations. The conclusions of the present work are limited to the in vitro conditions of the study and should be confirmed by further in vivo investigations.

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