

# Comparative Study to Evaluate the Antimicrobial Effect of MTAD, 17% EDTA, and 3% NaOCl- Against *Enterococcus faecalis* in Primary Teeth for Root Canals Therapy (in Vitro Study)

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## Abstract

**Aim:** This study presented to compare the antimicrobial effect of MTAD, 17% EDTA, and 3% NaOCl, against *Enterococcus faecalis* (*E. faecalis*).

**Methodology:** The study utilized 40 extracted human primary teeth with single root canal. The samples were instrumented and sterilized, and then, these teeth were infected with *E. faecalis* for 2 weeks, after that tooth were divided randomly into 2 groups according to the irrigants used. All the samples were incubated in brain heart infusion (BHI) broth. Irrigation processes were completed according to each material instruction. The effectiveness of irrigants was evaluated by comparison between the pre-irrigation and post- irrigation samples using serial dilution (CFU/ml).

Statistical analysis was done using one-way anova with dunnett t3 post hoc test.

**Results:** all samples treated with MTAD showed nearly complete absence of bacteria. the samples treated 3% NaOCl and 17% EDTA showed similar result to the first group, Statistical analysis of the data using dunnett t3 test showed there is no significant difference between the groups ( $P > 0.05$ ).

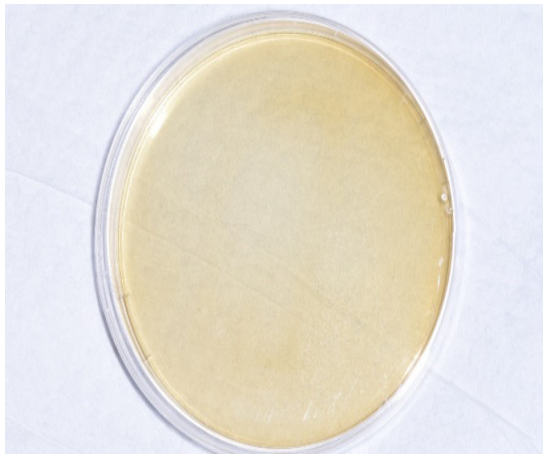
**Conclusion:** The study concluded that MTAD was effective as 3% NaOCl -17% EDTA against *E. faecalis*.

**Keywords:** BHI broth, *Enterococcus faecalis*, 17% EDTA, MTAD, 3% NaOCl, Primary teeth.

## Introduction

Microorganisms are playing a fundamental role in etiology of pulp and periradicular lesions [1]. Successful therapy of root canal relies on triad of instrumentation, disinfection and obturation [2]. The major determinant in the healing of periapical tissues is the disinfection of the root canal [1]. This is happen though the use of antimicrobials and chemo mechanical preparation, which are effective in reducing the bacterial load, some bacteria can still persist [3]. One among the facultative organism

is *Enterococcus faecalis*, which is persistently found in failures of root canal treatment [4], and is resistant to various intracanal medicaments [5]. The microorganisms, which found in the root canals of permanent teeth are similar to those in the root canals of deciduous teeth [6, 7]. Though, a commonly used root canal irrigant, is sodium hypochlorite (NaOCl), it has an unpleasant taste and odor it does not consistently system for disinfect the root canal [8], and is toxic, when extruded into the periradicular tissues [9]. So, a search for better root canal irrigant continues, because of these limitations.



**Figure 1: bile esculin blood agar plate.**

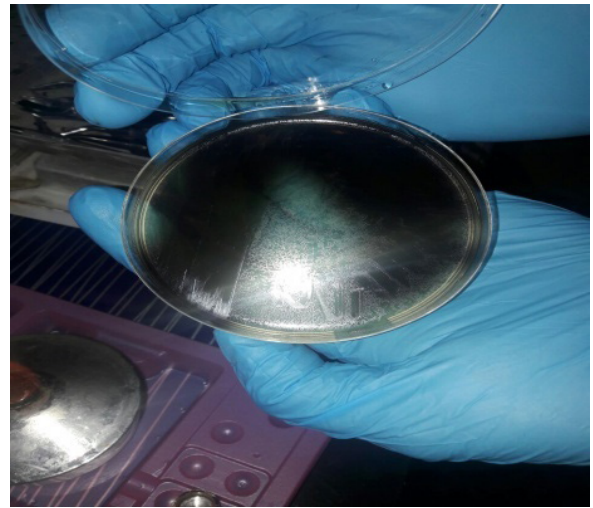
Torbinejad in 2003 introduced, a new irrigant, such as MTAD which is a mixture of Doxycycline, citric acid, and detergent (Tween-80)<sup>[7]</sup>, which is capable of safely removing the smear layer<sup>[10]</sup>, and eliminating *E.faecalis*<sup>[11]</sup>. The present study sought to compare the antimicrobial efficacy of MTAD, 3% NaOCl, and 17% EDTA against *E.faecalis*.

## Methodology

### Material and Method

Forty extracted single rooted of human primary teeth, were collected from children, aged 6-7 years, (Tow-third of the root length, at least should be presented). Crowns of teeth, were cut leaving only the roots. Determination of working length should be completed by radiograph. Instrumentation of the roots, to size 30 K-type file. Then cleaning the samples with normal saline, and sterilized by <sup>[12]</sup>.

*E.faecalis*, should be isolated from infected root canals. Many samples were taken from chronic infected roots that suspected to be inhabitant with *E.faecalis*. there was a special test called bile esculin test (selective test for *E.faecalis*) used for first identification of bacteria (figure 1) where, such a black deposit appears after 24 hours of samples culturing (figure 2). Then *E.faecalis*, further detecting by another way called vitek2 system for more accuracy (figure 3).

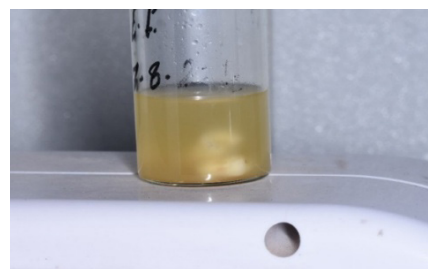


**Figure 2: E.faecalis (black deposit) in bile esculin agar**



**Figure 3: vitek 2 system**

The tooth specimens sterilized and then inoculated with *E.faecalis* that, after that was cultured in Brain-Heart Infusion (BHI) broth at 37°C. In order to inject the broth suspension inside root canal, 30 gauge needle was used. All specimens tooth must be immersed in broth at 37°C to allow bacterial growth (figure 4). Medium in this process should be changed once a week for each 4 respectively weeks. Two weeks' period was chosen for inoculation of bacteria, as recognizable number of colonies of bacteria has been produced after it. After this process, teeth were removed from the bacterial culture. Then we covered the root apices with Cavit™. After that, the end of each tooth specimen was wiped with 3% sodium hypochlorite in order to disinfect the outside of the tooth before irrigation treatment <sup>[13]</sup>.



**Figure 4: teeth specimens in brain heart infusion broth.**

Figure6: colonies of *E.faecalis* in the blood agar plate.

Then, all samples were divided randomly in to 2 groups, for experimental procedure of irrigation materials. Paper point that used for taking swap should be sterile, this swap from root canal (pre samples) before irrigation procedures. Importantly, we must be irrigated the root canal with normal saline and then preoperative swap was taken; normal saline here acts as good media for inoculation the content of canal.

**Irrigation procedure:**

Group 1: In this group, 20 roots samples, each root canal was irrigated firstly with 2ml 3% NaOCl, then normal saline cleaning, followed by irrigation with 17% EDTA, and final irrigation with normal saline<sup>[14]</sup>.

Group 2: In this group, 20 samples irrigated with MTAD, 2 ml of MTAD used for root canal irrigation, followed by irrigation with normal saline <sup>[15]</sup>.

Sterile paper point, used for taking post irrigation samples from the root canal. Both pre and post sample, are kept in Eppendorf tubes, special tubes, which containing 1ml of normal saline, (figure 5) and then transferred to the laboratory, for serial dilution, and bacterial count by using cfu/ml procedure (figure6).

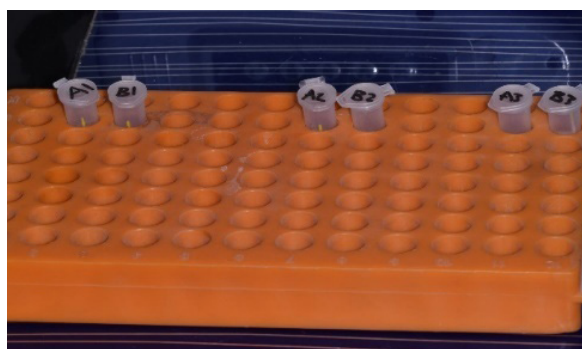


Figure 5: pre and post irrigation samples.



Figure6: colonies of *E.faecalis* in the blood agar plate.

**Results**

In this study the number of bacterial colonies in both irrigation regimens are reduction as in table (1).

**Table 1: The values of antibacterial effect of the Tow irrigant methods used in the study**

Groups	Mean	Std. Deviation	Minimum	Maximum
MTAD	98.037	1.029	95.45	100.00
NAOCL&EDTA	99.187	1.975	94.00	100.00

Result of one way anova revealed that there is no significant difference between antibacterial efficacy of both groups  $p > 0.05$  shown in (table 2).

**Table 2: Statistical values of one wayanova analysis for both Groups**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5832.821	2	2916.410	240.724	.161
Within Groups	508.837	42	12.115		
Total	6341.658	44			

Levene statistics=17.993, df=2, p value=0.161.

**Table 3: The antibacterial effect of the irrigant methods by Dunnett t3 post hoc test, Comparison Study.**

Comparisons between groups using Dunnett T3					
(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
MTAD	NaOCL&EDTA	-1.151	.161 [NS]	-2.636	.335

There is no significant difference showed in comparisons between MTAD and 3% NaOCL – 17% EDTA group, the p value = 0.161 ( $p > 0.05$ ). **Table 4: The antibacterial effect for pre and post samples for both groups**

Groups	Paired Samples Statistics				T value	P value	Effect size
	PRE		Post				
	Mean	SD	Mean	SD			
MTAD	3.675	1.251	.067	.032	11.202	.000[HS]	2.892
NaOCL& EDTA	2.041	.626	.014	.034	12.394	.000[HS]	3.200

Df = 14

In this study, result showed the reduction in the means percentage of the post samples of bacterial colonies for both methods, as in table 4, but the large effect size is 2.892 in the MTAD group, and (3% NaOCL-17% EDTA) group is 3.200 effect size, as in table 4.

### Discussion

In order to eliminate the bacteria and their products, and the substrate from the root canal system of teeth, doing root canal treatment<sup>[16]</sup>, so this process is the main aims for our study. In this process, the use of irrigation solution is essential to ensure bacterial elimination, and removal of organic tissue pieces<sup>[17]</sup>. Maximum tissue dissolving effect on the necrotic tissues, when we have maximum anti-bacterial effect of irrigant, and the least toxic effect on the peripheral tissues are some important features of an ideal root canal irrigant<sup>[18]</sup>. Primary teeth have the complex morphology, and the irregularity of the root canals, which of negatively affect the success of chemo-mechanical endodontic treatment<sup>[19]</sup>. The most commonly employed root canal irrigant is sodium hypochlorite, till now<sup>[20]</sup>, the antimicrobial activity of NaOCl is by the release of hypochlorous acid (HOCl), oxidative action of this acid on sulfhydryl

groups of bacterial enzymes, by the action of (HOCl) will disrupted the metabolism of the microorganism<sup>[20]</sup>. Sodium hypochlorite, it is an effective antibacterial agent, but NaOCl is toxic when extruded to the periradicular tissue<sup>[18]</sup>. Many primary teeth needed treatment for root canal, permanent tooth follicles can be damaged by NaOCl during treatment, and also peripheral tissues and oral mucosa. Therefore, a new irrigants should be found, research for that must be continues. MTAD, new material introduced in 2003, it was subjected to various test procedures, in order to evaluate its efficacy, it was compared with various commonly used irrigants. The antimicrobial activity of both MTAD over 3% NaOCl seen in this study are in conformity with the findings of Shabahang, and Torabinejad's study<sup>[11, 21]</sup>.

The most important organism in this study was *E. faecalis*. Therefore, it was selected, because it is most commonly isolated in endodontic retreatment of apical periodontitis<sup>[4]</sup>, sodium hypochlorite<sup>[20]</sup>, potassium iodide<sup>[22]</sup> or calcium hydroxide<sup>[23]</sup> chemical material irrigants had been identified to have a resistant currently when used, and has been found to survive as a mono-infection in root canals<sup>[2]</sup>. There is many previous in vitro studies, which have shown a high level of susceptibility

of *E. faecalis* to MTAD, this solution was diluted 200 times and still has the same efficacy of, while NaOCl loses its antibacterial activity against the *E. faecalis* beyond 32 times dilution<sup>[11]</sup>.

MTAD, its acting by removing the smear layer of bacteria with significantly less erosion of the dentinal tubules in comparison with EDTA(10). MTAD, when was evaluated for biocompatibility, it was prove to be less cytotoxic, and more safer than Eugenol, Ca(OH)<sub>2</sub> paste, 5.25% NaOCl, 3% H<sub>2</sub>O<sub>2</sub>, 0.12% Chlorhexidine gluconate<sup>[24]</sup>. The antimicrobial effectiveness of MTAD, by anticollagenase activity of Doxycycline, which it causes low Ph and have ability to be released gradually over time<sup>[10]</sup>, also its action by citric acid was facilitated the removing the organic and inorganic substances. Surface tension of solution reduces on the dentinal tubule by Tween-80, therefore, allows Doxycycline to penetrated more deeper into the tubules. Capacity of MTAD to kill *E. faecalis* after a mere exposure of 5 minutes, which is one of the significant features of this material, making it useful in the clinical situation. However, this effect was not seen with NaOCl<sup>[25]</sup>. Newberry et al. showed that MTAD killed most strains of *E. faecalis* when diluted 1:512 times and inhibited most strains of *E. faecalis* growth when diluted 1:8192 times<sup>[26]</sup>. Thus MTAD has ideal properties for root canal irrigant. The results of the our study was identical to the findings of Portenier et al<sup>[27]</sup>, Ghoddsi et al<sup>[28, 29]</sup>, and Davis et al<sup>[30]</sup>, while studies done by Dunavant et al<sup>[29]</sup>, Baumgartner et al<sup>[30]</sup>, Krause et al<sup>[31]</sup> are differences with these results. These differences in the results due to differences in our methodology and variance in strains tested, and the modification in our processing.

### Conclusion

Conclusion based on the data of the present study, biopure MTAD has an effectiveness as 3%NaOCl- 17% EDTA. However, more in vitro and in vivo studies are important to prove the using of MTAD as good irrigant against *E. faecalis*.

**Conflict of Interest** – Nil

**Source of Funding**- Self

**Ethical Clearance** – Not required

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