COMPARISON OF THE EFFECT OF CALCIUM HYDROXIDE 
AND THE MODIFIED TRIPLE ANTIBIOTIC PASTE AS INTRACANAL 
MEDICATION ON THE ELIMINATION OF \textit{ENTEROCoccus \textit{FAECALIS}} IN RETREATMENT CASES. A RANDOMIZED 
DOUBLE BLIND CLINICAL TRIAL

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\textbf{ABSTRACT}

\textbf{Introduction:} The present study aimed to evaluate the effect of calcium hydroxide and the modified triple antibiotic paste (MTAP) as intracanal medication on the elimination of the \textit{Enterococcus faecalis} in retreatment cases.

\textbf{Methods:} Sixty patients who attended the outpatient endodontic clinic, faculty of oral and dental medicine, Cairo University were included in this research. Inclusion was limited to patients with symptomatic root canal treated teeth. All teeth underwent non-surgical retreatment. After removal of the gutta percha using protaper rotary retreatment files and irrigation with sodium hypochlorite, the canals were dried and one of the studied medicaments was inserted into the canal in a random sequence. The study was divided into 2 equal groups: Calcium Hydroxide group (CH group n=30) and modified triple antibiotic paste group (MTAP group n=30). Before application of the medicament, canals were sampled using paper points and the colony-forming units grown were counted. After 7 days, the medicaments were removed and the same sampling procedure was repeated. Obturation was then carried out.

\textbf{Results:} For both groups, significant decrease in the colony forming units (CFU) of the \textit{Enterococcus faecalis} resulted after material application. There was no significant difference in CFU between CH group and MTAP group after medication application.

\textbf{Conclusions:} MTAP application resulted in decreased CFU of \textit{Enterococcus faecalis} in nonsurgical retreatment endodontic cases. However there was no statistical significant difference between the use of calcium hydroxide and the modified triple antibiotic paste as intracanal medicament.

\textbf{KEYWORDS:} Calcium hydroxide, modified triple antibiotic paste, \textit{Enterococcus faecalis}, retreatment

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INTRODUCTION

Microorganisms have been well known to play an important role in pulpal and periapical diseases. Tzanetakis et al. suggested that persistent infections may have more diverse bacterial communities than primary infections (1). Eradication of causative microorganisms during root canal treatment procedures helps to attain successful results. Because of the complex nature of the root canal system and the presence of many inaccessible areas, lateral and accessory canals, apical deltas and isthmus, a combination of mechanical instrumentation and irrigation is necessary to decrease the amount of bacteria/micro-organisms in the root canal system (2).

It is believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth (3). Enterococcus faecalis despite of making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periodontal lesions after root canal treatment. They are gram positive cocci that occur singly, in pairs, or as short chains and able to survive in the root canal as a single organism or as a major component of the flora. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen and can penetrate deeply into the dentinal tubules and resist bacitracidal substances commonly used in endodontic procedures (4). Thus intracanal medication is needed in the cases where bacteria are resistant to routine treatment, and where the therapy cannot be successfully completed due to the presence of pain or continuing exudates (5). Calcium hydroxide is the most widely used intracanal medication due to its many properties as an ideal root canal dressing. It has an alkaline pH, bactericidal and neutralizes the remaining tissue debris in the root canal system. Calcium hydroxide also promotes an alkalinizing osteogenic environment on the surrounding tissues through the continuous release of OH- ions (6).

Antibiotics can be used as an adjunct to endodontic treatment in a number of ways locally (intracanal), systemically and prophylactically (7). Because of the complexity of the root canal infection, it is unlikely that any single antibiotic could result in effective sterilization of the canal. A combination would be needed to address the diverse flora encountered. The most commonly used medicament is a combination of three antibiotics, referred to as a triple antibiotic paste (TAP). This formulation was first used by Sato et al. and contains metronidazole, ciprofloxacin, and minocycline (8).

Metronidazole is a nitroimidazole compound. It is selectively toxic to anaerobic microbes. It also exhibits broad spectrum antimicrobial activity against protozoa and anaerobic bacteria. Tetracycline, which includes doxycycline and minocycline are primarily bacteriostatic, inhibiting protein synthesis by binding to 30S ribosomes in susceptible organisms. They exhibit broad spectrum of activity against gram positive and gram negative microorganisms (9). Ciprofloxacin is a synthetic floroquinolone with rapid bactericidal action. It exhibits very potent activity against gram negative bacteria but very limited activity against gram positive bacteria. Most of the anaerobic bacteria are resistant to ciprofloxacin. Hence it is often combined with metronidazole in treating mixed infections (10). However, crown discoloration has been associated with TAP (11). Therefore, recent studies have suggested substituting minocycline with another antibiotic (12, 13). Clindamycin has been found to be effective against various endodontic pathogens (14). A modified triple antibiotic paste (MTAP) composed of metronidazole, ciprofloxacin, and clindamycin was successfully used as an intracanal medicament to disinfect necrotic immature teeth during an endodontic regeneration procedure (15). The aim of this study was to compare the antibacterial effect of calcium hydroxide and MTAP on the elimination of Enterococcus faecalis in retreatment cases.
MATERIALS AND METHODS

The outline of this study was approved by the Ethical Committee of Cairo University. This study was performed over a 5-month period spanning from September 2016 till January 2017.

All the patients who participated in this study were informed about the treatment protocols, benefits, risks, treatment alternatives, and signed an informed consent.

Sample size

The study was planned with 30 experimental subjects (MTAP) and 30 control subjects (CH). A total sample size of 52 patients was sufficient with a power of 80%, and a significance level of 5%. This number has been increased to a total sample size of 60, to allow for losses of around 15%.

Randomization and blinding

The sequence generation was done for the patient’s numbers (from 1 to 60) using computer sequence generation (http://www.random.org/) which gave a table for group A and group B with randomized patients’ numbers (30 number in each group). Group A (control) calcium hydroxide intracanal medication group (CH) while group B (intervention) was the modified triple antibiotic intracanal medication group (MTAP). For the allocation concealment mechanism, 60 papers (each was eight folded) with a number from 1 to 60 were placed inside opaque envelopes. Each patient picked up an envelope before the treatment. The number in the envelope determined which intracanal medication was to be used for that patient according the table generated by the computer. The operator knew which intracanal medication will be used but the assistant who counted the CFU and the patient were blind.

Trial design

The study was reported following the guidelines provided in the Consolidated Standards for Reporting Clinical Trials (CONSORT) statement. The present was a parallel-group randomized controlled clinical trial with an allocation ratio of 1:1.

Participants

**The inclusion criteria were:**
- 18 years old or older
- Single rooted restorable teeth with a single root canal diagnosed as symptomatic root canal treatment failure within the past six months.
- Clinical diagnosis of symptomatic previously treated root canal: presence of severe or sharp throbbing pain or dull aching pain, and pain on biting. Percussion test was positive and the radiographic examination revealed short or defective root canal treatment, normal periodontal structure
- Patients who were able to sign an informed consent form
- Subjects classified as ASA-1 following the classification proposed by the American Society of Anesthesiologists

**The exclusion criteria were:**
- Patients with vital teeth, trauma, root resorption, immature/open apex, or a root canal in which the patency of the apical foramen could not be established
- Presence of diffuse or localized swelling and the presence of sinus tract
- Patients taking antibiotics in the seven days before intervention

Interventions

All subjects were treated in the out-patient endodontic clinic, faculty of oral and dental medicine, Cairo University. Data were collected and analyzed in the same department. All patients were treated by a single experienced operator. Intervention was performed on two visits.
First visit:

Patients were anesthetized using nerve block local anesthesia or infiltration local anesthesia (Carpule Mepecaine-L, Alexandria Company for Pharmaceuticals and Chemical Industries, Alexandria, Egypt) according to the tooth location in mandibular or maxillary arch respectively. Previous coronal restoration was removed. the teeth were isolated with rubber dam and both were disinfected with 30% H_2O_2 (volume/volume [V/V]) for 30 seconds, followed by 2.5% NaOCl for the same period of time and 5% sodium thiosulfate was used to inactivate the NaOCl. Gutta percha removal was done using protaper rotary retreatment files (DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN) in the following manner D1 has a cutting tip to facilitate initial penetration into the filling material (coronal third). D2 and D3 both have non-cutting tips and were used to remove material from the middle and apical thirds, respectively using guttapercha solvent (Carvene, PREVEST DENPRO LTD, Jammu, India.). A periapical radiograph was taken to ensure that all the gutta percha was removed. Working length was determined using an electronic apex locator (Root ZX, J.Morita USA, Irvine, CA.) then confirmed with intraoral periapical radiograph, to be 0.5-1 mm, shorter than radiographic apex. Cleaning and shaping was done using crown down preparation technique with the use of ProTaper Universal (PTU; Dentsply Maillefer, Ballaigues, Switzerland) in an endodontic motor according to the manufacturer instructions (X-Smart, Dentsply Tulsa, Salzburg, Austria.). The canals were thoroughly irrigated using 3ml of 2.5% sodium hypochlorite between every subsequent instrument file using 30-gauge Max-i-Probe syringe. Glyde file prep (Dentsply, Maillefer, Ballaigues, Switzerland) was used as a lubricant during mechanical preparation. Depending on each tooth the final instrumentation size was ProTaper F3 or F4. A final irrigation was performed for both groups using 1 ml of 0.85% sterile saline solution. Canals were then sampled using paper points (Protaper Paper Points) (DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN). After each sampling, paper points were transferred to tubes containing 1 ml of brain heart infusion (Oxoid Limited). After 10-fold serial dilutions 50 microns of each dilution was incubated on Bile Esculin Agar (HiMedia Laboratories) at 37°C for 48 hrs. The colony-forming units grown were counted. Using Lentulo Spiral (DENTSPLY, LTD, CANADA.) medicaments were placed under aseptic conditions into the canals according to each group. Modified triple antibiotic paste group (MTAP group n=30) consisted of 1 mg/mL MTAP, (1 g of USP-grade antibiotic powders comprising ciprofloxacin 14%, metronidazole 43% and clindamycin 43% (Skywalk Pharmacy, Wauwatosa, WI, USA) were mixed with 1 ml of sterile water then 100mg of the compound powder dissolved in 100ml of sterile water, then 8g methyl cellulose powder (Methocel60HG; Sigma-Aldrich, St.Louis, MO, USA) was added to 100ml of each 1mg/mL of MTAP solution under magnetic stirring for 2 hours). Calcium hydroxide group (CH group n=30) consisted of 100mg calcium hydroxide powder mixed with 1ml of distilled water (PREVEST DENPRO LTD, Jammu, India). The access cavity was then closed with a temporary filling (MD-Temp, META BIOMED CO., LTD, Cheongju, Korea).

Second visit:

After 7 days, rubber dam was placed, the temporary filling was removed and master apical file was introduced in each root canal to loosen medicament and to create a space for subsequent irrigant, then each root canal was washed with 5 mL of sterile saline. Dryness of the canals by paper points was done and the same sampling procedure was repeated. The canal was irrigated with 1 mL of 20% citric acid (Ultradent® Citric Acid 20% IndiSpense® Syringe U.S.A.) and again irrigated with 5 mL sterile saline. Obturation was carried
out using the modified single cone technique with protaper guttapercha cones and resin sealer (ADSEAL, META BIOMED CO., LTD, Cheongju, Korea) with spreader (Mani, INC, Utsunomiya Tochigi, Japan) size according to master cone size, its depth short 2 mm of the working length. After obturation, a cotton pellet was placed in the pulp chamber and the access cavity was closed with a temporary filling to avoid coronal leakage.

**Outcome**

The reduction of *Enterococcus faecalis* from the root canals after application of the intracanal medications was measured. This was performed by comparing the number of colony-forming units of grown after each visit.

**Statistical analysis**

Data presented as means and standard deviation (SD) values. Data explored for normality using Kolmogorov Smirnov test. Data showed nonparametric distribution so; Mann Whitney U-test was used to compare between the calcium hydroxide and Triple antibiotic on mean CFU. Wilcoxon singed rank test was used to compare between before and after medication application for each material. The significance level was set at \( P \leq 0.05 \). Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows.

**RESULTS**

Patients’ flow diagram is showed in Figure 1.

1. **Effect of each medication after application on CFU:**

Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different follow-up for different medication presented in

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Fig. (1) Consort flowchart to depict enrolment of patients, allocation to intervention, and posttreatment analysis.
table (1). For all the tested materials, significant decreases in CFU resulted after material application for all the different concentrations.

2. Difference between tested groups before application on CFU:

Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different tested groups before medication presented in table (2) and figure (2). For all the tested groups, insignificant difference in CFU before medication application.

3. Difference between tested groups After application on CFU:

Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different tested groups after medication presented in table (3) and figure (3). For all the tested groups, insignificant difference in CFU after medication application.

TABLE (1) Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different follow-up for different medication.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Time</th>
<th>Before Medication placement</th>
<th>After Medication placement</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Calcium Hydroxide</td>
<td>1/10</td>
<td>9.40</td>
<td>7.99</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>3.50</td>
<td>3.51</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>1/1000</td>
<td>1.40</td>
<td>1.94</td>
<td>1.00</td>
</tr>
<tr>
<td>Modified Triple Antibiotic Paste</td>
<td>1/10</td>
<td>8.59</td>
<td>11.48</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>2.69</td>
<td>2.24</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1/1000</td>
<td>2.27</td>
<td>8.13</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NS= Non-significant, *=Significant

TABLE (2) Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different tested groups before medication.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Before Medication placement</th>
<th>Calcium Hydroxide</th>
<th>Modified Triple Antibiotic Paste</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Calcium Hydroxide</td>
<td>1/10</td>
<td>9.40</td>
<td>7.99</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td>1/100</td>
<td>3.50</td>
<td>3.51</td>
<td>2.50</td>
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<tr>
<td></td>
<td>1/1000</td>
<td>1.40</td>
<td>1.94</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NS= Non-significant, *=Significant
In most of the failed cases of root canal treatment the Gram +ve facultative anaerobe Enterococcus faecalis is the frequently isolated microorganism (17). This is due to its unique capability to invade dentinal tubules depth and surviving extreme periods of pH and limited nutrition. In the present study calcium hydroxide was used as it is the commonly used intracanal medicament. Its effectiveness is linked to the diffusion of hydroxyl ions through the dentinal tubules and accessory canals into areas where bacteria and their byproducts may be harbored. In addition to acting as a physical barrier, the calcium hydroxide dressing may both prevent root canal re-infection and interrupt the nutrient supply to the remaining bacteria (18-21). Several reasons have been proposed to explain why E. faecalis is able to survive intracanal calcium hydroxide. E. faecalis passively maintains pH homeostasis using its proton pump and is unable to survive pH of 11.5 and greater (22-25). Antibiotics could be one of the current treatment modalities proposed in eliminating E. faecalis from the root canal system. The modified triple antibiotic paste was used in this study rather than the triple antibiotic paste, as minocycline was substituted by clindamycin to avoid teeth discoloration caused by the tetracycline isomer (26).

### DISCUSSION

TABLE (3) Mean, standard deviation (SD), Median, Range, Min., and max. for the CFU results of different tested groups After medication.

<table>
<thead>
<tr>
<th>Medication</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th>Maximum</th>
<th>Minimum</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Hydroxide</td>
<td>1/10</td>
<td>5.52</td>
<td>6.40</td>
<td>3.00</td>
<td>28.00</td>
<td>28.00</td>
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<td>1.13</td>
<td>1.55</td>
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<tr>
<td></td>
<td>1/100</td>
<td>2.21</td>
<td>4.39</td>
<td>1.00</td>
<td>20.00</td>
<td>20.00</td>
<td>0.00</td>
<td></td>
<td>0.27</td>
<td>0.64</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1/1000</td>
<td>1.10</td>
<td>2.96</td>
<td>0.00</td>
<td>12.00</td>
<td>12.00</td>
<td>0.00</td>
<td></td>
<td>0.03</td>
<td>0.18</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

NS= Non-significant, *=Significant

Fig. (2) Box and plot showing the CFU for different groups before medication application.

Fig. (3) Box and plot showing the CFU for different groups after medication application.
The modified triple antibiotic paste concentration used was 1 mg/ml, where it was found that the recommended dose of MTAP is from 0.1-2mg/ml to prevent the cytotoxic effect, it was also found that only 1mg/ml was able to eradicate 99.9999% of established *E.faecalis* biofilm \(^{(27,28)}\). Protaper universal retreatment files were used to remove old gutta percha present inside the canals, where it was found to be the safest, fastest and most efficient way to remove gutta percha than using gates glidden and hedstrom files \(^{(29)}\). The intracanal medications were applied for 1 week in both groups. Siqueira et al 2007 stated that the application of calcium hydroxide for 1 week was sufficient to reduce the canal bacteria to a level that gave a negative culture \(^{(30)}\). The results of the current clinical investigation showed reduction in the CFU after applications of both medicaments. But there was no statistically significant difference in the reduction of the *Enterococcus faecalis* when dressed with either intra canal medicaments even though MTAP resulted in less CFU in comparison to the calcium hydroxide. Despite the effectiveness of calcium hydroxide in eliminating a wide range of microorganisms, our results support other findings showing that calcium hydroxide could not completely eliminate *E.faecalis* \(^{(31-33)}\). This could be due to the dentine buffer properties as it might decrease the PH effect of the calcium hydroxide. *E.fecalis* will not be eliminated even in an environment with a pH 11.5. Moreover, the interaction between dentine and *E.faecalis* may result in bacterium resistance \(^{(34)}\). The decrease in CFU as a result of MTAP application is due to the high antibacterial effect on *E.fecalis*. Combination of these three antibiotics overcomes bacterial resistance and achieves higher antimicrobial action. Individually, ciprofloxacin has broad spectrum activity and acts against both Gram-positive and Gram-negative bacteria by inactivating enzymes and inhibiting cell division. Metronidazole is effective against obligate anaerobes, which are common in the deep dentin of infected root canals and acts by disrupting bacterial DNA. Clindamycin has a primary bacteriostatic effect. It is a bacterial protein synthesis inhibitor. The incomplete elimination of *E faecalis* could be attributed to the combined spectrum of antimicrobial activity and synergetic or additive actions of antibiotics ciprofloxacin, metronidazole, and clindamycin found in MTAP. Also, it may be attributed to the antagonism between bactericidal and bacteriostatic antibiotics as ciprofloxacin is a bactericidal antibiotic and clindamycin is a bacteriostatic antibiotic \(^{(35)}\).

In conclusion MTAP application resulted in decreased CFU of *Enterococcus faecalis* in non-surgical retreatment endodontic cases. However there was no statistically significant difference between the use of calcium hydroxide and the modified triple antibiotic paste as intracanal medicament

**ACKNOWLEDGMENT**

The authors deny any conflicts of interest

**Funding**

This study is self-funded

**REFERENCES**


