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# ANTIBACTERIAL EFFECT OF VARIOUS IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS

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## INTRODUCTION

Complete disinfection of the root canals with a combination of chemical agents and root canal files plays an important role in the success of root canal treatment.

Although, treatment is adequate, failure may occur given the presence of pathogenic residual bacteria in dentinal walls of the canal. Complex anatomy of root canal systems, with a variety of dentin tubules, apical canal ramifications, isthmuses and irregularities where bacteria may exist in the form of biofilms, makes the elimination of the microbial environment difficult. These microorganisms may reinfect the root canals if they cannot be eliminated.

Among the microorganisms, commonly isolated from root canals, Enterococcus faecalis (E. faecalis) has the ability to penetrate the dentinal tubules, exhibits strong adhesion to collagen and shows resistance to the irrigation solutions usually used during the instrumentation of root canals. And also, it is the representative bacteria of secondary infections in its planktonic and biofilm forms.

During and after instrumentation, the irrigants facilitate the removal of any residual tissue, microorganisms, and dentin debris from the root canal. A number of agents have been used as irrigation solution: NaOCl, ethylenediaminetetraacetic acid (EDTA), chlorhexidine.

However, due to the potential side effects, safety concerns, and limitations in instrumentation methods there is difficulty in complete cleaning of root canals, making it necessary to develop alternative procedures to optimize the cleaning process. So, researchers are in search of new disinfection processes that will be effective for all bacteria and biocompatible with periradicular tissues in order to eliminate infection from root canal systems.

Ozone (O3) ‐ a natural pale blue gas that can be found in the atmosphere, is a powerful oxidizing agent that can be produced by generators. As an antimicrobial agent, O3 causes cell lysis – powerful bactericidal, virucidal, and fungicidal effects. Ozone can be used in various forms for the treatment of endodontic conditions such as Ozonated water, gaseous ozone and Ozonized oil.

Even though, there are many studies available regarding the use of ozonated water and gaseous ozone, there is no study exist in the literature regarding the use and efficacy of ozonized olive oil as an endodontic irrigant in comparison with other irrigants like Sodium Hypochlorite, EDTA and Chlorhexidine, in disinfection strategies in the elimination of E. faecalis in root canals. Considering the role of E. faecalis and its by products in the etiology of persistent pulpal and periapical pathogenesis, the aim of this study was to evaluate the efficacy of different irrigants in comparison with O3.

## MATERIALS AND METHOD

The present in-vitro study was conducted to compare the antibacterial inhibitory effect of ozonated olive oil and sodium hypochlorite, EDTA and Chlorhexidine on Enterococcus faecalis using agar well diffusion test.

40 single rooted with mature apices were randomly divided into 4 groups of 10 teeth each, depending upon the type of irrigant:

Group 1- 2.5% NaOCl ((Bharat Chemical, India).

Group 2- 17% EDTA (Prime Dental Products Pvt. Ltd, India)

Group 3- 2% Chlorhexidine solution ((Indoco remedies Ltd. Aurangabad, India)

Group 4- Ozonated olive oil (DentOzone, India) which is composed of medical‐grade ozonized herbal olive oil.

Selected teeth were de-coronated apical to the cement-enamel junction to standardize the length to 13mm with the diamond disc under water coolant.

Working length of all samples was established by size 10 K-file inserted into the canal until the tip of the file become visible at the apical foramen.

Enterococcus faecalis strains (ATCC 29212 strain) prepared in Department of Microbiology Dr BRAMC were incubated in the canals.

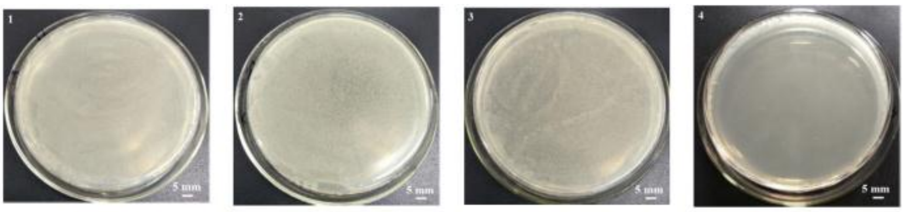
Root canal shaping procedure was performed with ProTaper rotarty files upto the size of F3. During cleaning and shaping root canals were irrigated with 5 mL irrigants in the each group with respective irrigants using a 27‑gauge dental injector with peristaltic motion that was placed 2 mm away from the WL for 2 min. The final irrigation was done with saline and the irrigant flown out from the apices of the tooth was collected for incubation.

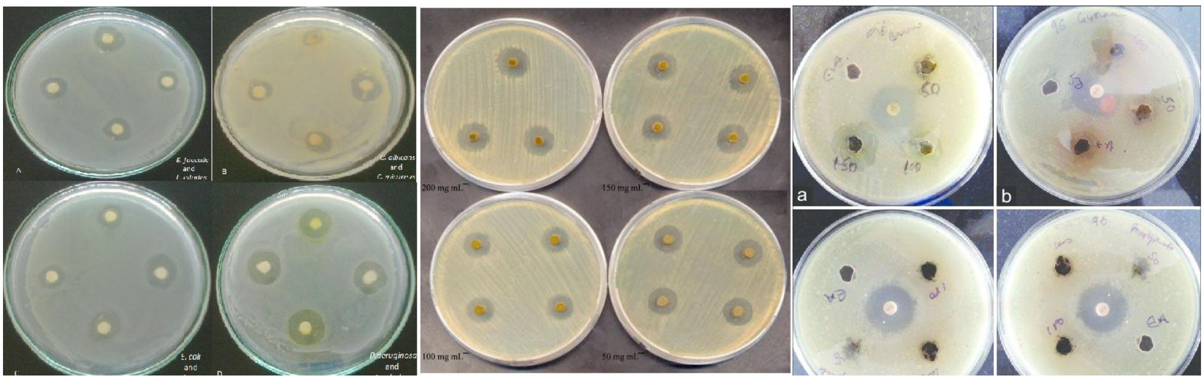
Agar well diffusion test was used to determine the antibacterial effect of the above mentioned irrigants against Enterococcus faecalis. The cultures were inoculated into sterile brain heart infusion (BHI) broth and incubated at 37°C and the turbidity of 0.5 Mcfarland standard was achieved. Sterile cotton swabs were dipped in the broth and streaked on the Brain Heart agar media plates. Sterile template was used to cut four wells (5mm in diameter) at equal distances in each agar plate.

In each plate, 50 µl of sodium hypochlorite solution (group 1), 50 µl of EDTA (group 2), 50 µl of Chlorhexidine solution (group 3) and 50 µl of ozonated olive oil (group 4) were placed in 4 wells using sterile micro pippettes.

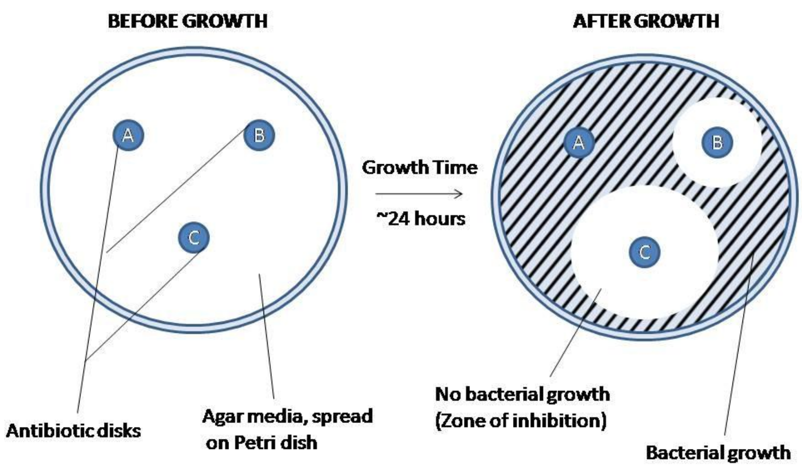
The plates were then incubated at 37°C for 24 h. The diameters of zones of bacterial inhibition formed around wells containing of test materials were measured and recorded after incubation for 24hr and 48hr. The diameter of the zone of inhibition of bacterial growth around the well was measured using ruler (in mm).

**AGAR PLATES**





**TEST SAMPLES AFTER INCUBATION**



## RESULTS

Statistical analysis was performed using SPSS software program (SPSS 18; SPSS, Chicago, IL, USA). Data were presented as mean ± standard deviation (mean ± SD). Diameters of the zones of bacteraial growth inhibition (in mm) were compared between both groups using independent t test. The level of significance was set at $p < 0.05$.

The mean values for the diameter of zones of bacterial inhibition for both groups after 24 h are shown in the following table.

| GROUPS | Mean diameter of inhibition zone (mm) | Standard deviation |
|---|---|---|
| 1 | 25.18 | ±1.396 |
| 2 | 13.28 | ±1.446 |
| 3 | 39.22 | ±1.148 |
| 4 | 45.46 | ±1.127 |
| p VALUE Between Groups | 0.00* | |

Significance level p<0.05, *significant

There was no change in the diameter of the inhibition zones after 48 hours.

## DISCUSSION

Irrigation is an integral part of chemo‑mechanical preparation and it plays a major role in the cleaning and disinfection of the root canal system. Irrigation renders the canal system free of necrotic pulp tissue, bacteria and dentinal debris.

The study simulated clinical conditions by using human teeth and microorganisms that were grown in a liquid medium containing a tooth, which are similar to those found in the oral environment. In the present study, the root canal system was contaminated with E. faecalis, a Gram‑positive facultative bacteria. It has been reported to be the most commonly identified species in root canals that result in failed endodontic treatment.

Because of the hardy nature of this bacteria, it can grow and survive as a monoculture under diverse conditions, including in nutrition‑depleted root canals. It seems to be the one most able to penetrate into dentinal tubules, leading to gross infection.

### NaOCl

NaOCl is currently the most widely used irrigating solution due to its superior antibacterial efficacy. NaOCl is highly toxic at high concentrations and tends to induce tissue irritation on contact. Its accidental injection beyond the root apex causes tissue reaction characterized by pain, swelling, hemorrhage, and in some cases the development of secondary infection and paresthesia. Other drawbacks of NaOCl comprise its unpleasant taste, ability to bleach clothes, corrosion of endodontic instruments, inability to remove the smear layer, and weakening effect on dentine.

NaOCl can create large zones of inhibition against E. faecalis. However, the data regarding the inhibitory concentration and application time of NaOCl against E.faecalis are not clear enough in the literature. In the present study, a significant effect was achieved with 2.5% NaOCl irrigation, much like in previous studies.

### EDTA

EDTA has not shown an obvious antibacterial effect against E. faecalis in many studies. This study indicated that the antibacterial activity of 17% EDTA against E. faecalis was weaker than that of the other four irrigants. As a chelating agent removing the inorganic components in the smear layer, EDTA could change the permeability of cell membrane; this is the mechanism for its antibacterial activity. Nevertheless, 17% EDTA has large surface tension and small permeability, which made it difficult to penetrate into the dentine tubules to kill the E. faecalis exhaustively.

### Chlorhexidine solution

Our studies showed that 2% CHX irrigant had significant action against E. faecalis. These results suggest that in terms of antibacterial activities, CHX might be more effective than NaOCl & EDTA. CHX is a positively charged hydrophobic and lipophilic molecule, its positively charged ions aid the adsorption of CHX into the dentine and generate residual effects on pathogens in the root canal. Furthermore, the positive charge of CHX also helps the CHX molecule bind to negatively charged phosphate groups on microbial cell walls and alter the cells' osmotic equilibrium to further destroy bacteria.

### OZONE OLIVE OIL

Ozone, a powerful oxidizing agent, has strong bactericidal, virucidal, and fungicidal effects making it a potential agent for root canal disinfection. Ozone is nontoxic to oral cells and very efficient against antibiotic resistant strains. Its effect increases in acidic pH unlike NaOCl whose rate of decomposition rapidly decreases from pH 11 - 7. Three fundamental forms of ozone application are: – (1) ozonated water, (2) ozonated olive oil, and (3) oxygen/ozone gas. Ozonated water and olive oil have the capacity to entrap and then release oxygen/ozone, constituting an ideal delivery system. Ozone, when dissolved in water, becomes highly unstable and rapidly decomposes, so it cannot be stored. In contrast, when it is dissolved in an oil base, it has a life span that could be measured in years. It chemically reacts with oil and forms long complex molecules. Hydrolysis of ozonized oil can generate hydrogen peroxide, aldehydes, and acetones. Kishore et al. evaluated the antibacterial activity of the ozonized oil, calcium hydroxide, and their combination against Enterococcus faecalis and concluded that ozonized oil was the most effective medicament. Pratyusha et al. evaluated the antibacterial activity of the ozonated olive oil and cold pressed neem oil against E. faecalis using the agar well diffusion method and concluded that ozonated olive oil was more effective. The results of the present study showed higher antibacterial effect of ozonated olive oil when compared to Chlorhexidine, sodium hypochlorite & EDTA solution. The values of the diameters of the bacterial inhibition zones did not change after 48 h. The bactericidal efficacy of ozone is based on its ability to formoxidated radicals, as a result of which the cell membranes get damaged by altering the osmotic stability and permeability. During the ozonolysis reaction, there is typically an increase in peroxide and acid values and a decrease in iodine values.

However, there are certain contraindications for the use of ozone including pregnancy, hyperthyroidism, hemorrhage, favism, severe anemia, thrombocytopenia, acute alcohol intoxication, recent myocardial infarction, hemorrhage in any organ and ozone allergy.

## CONCLUSION

The ozone oil irrigating solution used in the present study may able to niche a suitable place for it in dentistry as it may provide complete antibacterial environment in the infected root canal. Based on the results of this it can be concluded that NaoCl, EDTA, CHX, and O3can kill E. faecalis in the matured biofilm at different levels. Their antibacterial activity from strong to weak was O3, CHX, NaoCl and EDTA. Further, ozone oil irrigating solution has superior antibacterial activity with that of chlorhexidine solution in eliminating the aerobic microbial flora from infected root canals.

## SCOPE

Further studies are also necessary to evaluate the physiochemical properties of the ozone oil irrigating solution to improvise its efficacy in vivo.

## LIMTATIONS

1. The bactericidal efficacy of ozone is based on its ability to form oxidated radicals.
   Ozonated olive oil - ozonolysis reaction is meaningless if peroxide released by ozonated oil could not be quantified. These considerations bring us to two important conclusions.
   First, ozonated oil manufacturers should describe the peroxide, acid, and iodine values on the product label.
   Second, further studies considering the standardization of ozonolysis procedures for vegetable oils are warranted.
2. Irrigants are provided in liquid form, presumably to facilitate penetration into the accessory canals and tubules whereas ozonated olive oil is more viscous in nature thereby limiting the penetration.