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Antimicrobial effectiveness of Endoactivator against Enterococcus faecalis in comparison to conventional and side vented needle irrigation: An *in vitro* study

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INTRODUCTION:

The complexity of the canal system makes it impossible to fully shape and clean with the endodontic instruments alone should be combined with disinfection protocol. As a result, Root canal disinfection is an exceedingly tough process that results in vast exposed regions and residual biofilms of bacterial species in the root canal.

AIM:

Using microbial collection and culture methods, the study investigated the effect of different irrigation systems on the reduction of *Enterococcus faecalis (E. faecalis)*

Materials and Methods:

E. faecalis was inoculated into sterilized human teeth. To irrigate the samples 30-gauge double-vented needle, an Endoactivator, and a conventional needle were used. After chemomechanical treatment, dentin fragments from the apical third of the canal were collected by Gates Glidden 1. Estimated colony forming unit (cfus) and incubation of E. faecalis per mg dentin after addition of 1 mL of sterile Brain infusion (BHI) suspension for the sample.

RESULTS:

The decrease in *Enterococcus faecalis* was seen in the experimental group compared to other groups ($p < 0.05$). Group I and Group III displayed a significant difference ($P<0.05$). CFU/ml counts between all three groups showed statistically significant differences

CONCLUSION:

The Endoactivator and double-side vented needle were better in bacterial reduction compared to conventional needle irrigation.

KEYWORDS:

Enterococcus faecalis, Endoactivator, Irrigation, Side vented needle

INTRODUCTION:

The three-dimensional root canal system shaping, cleaning, and filling was successfully completed (Do and Gaudin, 2020)(Siddique *et al.*, 2020). The complexity of the canal system makes it impossible to fully shape and clean with the endodontic instruments alone should be combined with disinfection protocol (Sairaman *et al.*, 2024; Swathi *et al.*, 2024). As a result, Root canal disinfection is an exceedingly tough process that results in vast exposed regions and residual biofilms of bacterial species in the root canal. (Gomes, Aveiro and Kishen, 2023)(Kamath *et al.*, 2022) However, when standard rotary and manual instruments are employed, approximately 35% of the instrumented root canal area remains unaltered due to the complex anatomy of the root canal. To permit cleaning in areas that were not mechanically treated and beyond the reach of root canal instruments, irrigation is a crucial part of root canal treatment. (Wigler *et al.*, 2023) The study

investigated the effect of the Irrigation systems on the reduction of *Enterococcus faecalis* using microbial collection and culture methods.

It is recommended to use alternative irrigation solutions after shaping to dissolve the inorganic and organic components using a deproteinizing agent (NaOCl) and a calcium-chelating agent (EDTA). (Mancini *et al.,* 2021) (Janani *et al.,* 2020). It is believed that Enterococcus faecalis is a particular opportunistic infection that causes chronic periapical disease. In the endodontically treated canal bacterial load will be lowered/nill, but the untreated canal will harbour more microbial flora .(Iandolo *et al.,* 2023)(Kamath *et al.,* 2022). Furthermore, the *Enterococcus faecalis* and *Porphyromonas gingivalis* species in the dentinal tubules are the primary causes of recurrent periradicular pathosis, having a 500-micron permeability, limiting the efficiency of automated irrigation in reducing *Enterococcus faecalis* bacteria. As a result, adequate penetration of the irrigant is necessary for effective debridement and disinfection, particularly in areas that are untouched by rotary instruments. (Badami *et al.,* 2023)

In the conventional needle irrigation method, solutions are administered only up to a distance of 1.1 mm beyond the needle tip, specifically targeting areas like the apical third. This limited delivery may lead to the entrapment of gas particles, potentially causing a vapor lock effect. (Virdee *et al.,* 2018) This falls short of adequately cleansing the intricate anatomy of the root canal system, encompassing lateral canals, isthmuses, fins, and accessory canals. (Mancini *et al.,* 2013) The limitations of CNI have led to the development of several manual and machine-assisted Irrigant Activation Techniques (IAT), some of the most well-known and researched of which are Manual Dynamic Activation (MDA), Passive Ultrasonic Irrigation (PUI), Sonic Irrigation (SI), and Apical Negative Pressure (ANP). (Virdee *et al.,* 2018) The EDDY sonic irrigation activation system, made of versatile polyamide and functioning at frequencies ranging from 5000 to 6000 Hz, is activated by an air-driven handpiece known as the Air Scaler, manufactured by VDW in Munich, Germany. The device is supposed to produce a three-dimensional movement that initiates Acoustic streaming and Cavitation, according to the manufacturer. (Urban *et al.,* 2017)

Therefore, The present research aimed to assess the actual benefits of utilizing activation devices as a substitute for manual irrigation techniques *Enterococcus faecalis* is considered a strong bacterial species that may persist within failed root canal treatment. (Alghamdi and Shakir, 2020) Therefore, this study aimed to evaluate the effectiveness of an irrigation system in reducing the amount of *Enterococcus faecalis* present. According to the null hypothesis under investigation, there would be no discernible difference in the decline in *Enterococcus faecalis* when various irrigation techniques were compared to conventional needle irrigation (SNI).

MATERIALS AND METHODS

Following ethical clearance from the institutional ethical council (IHEC/SDC/ENDO-1845/21/149), an in-vitro investigation was organized. The power for this investigation was set at

85%, and Alpha 0.05 was used to determine the sample size at an effect size of 0.55. A minimum of forty-five samples were used to create the experimental and control groups. (Toljan *et al.*, 2016) A total of 45 excised mandibular premolars with fully developed apices were chosen, extracted, and preserved in saline solution. Radiography was performed to ensure that there was just only single canal in each tooth. (Miller and Baumgartner, 2010) Excluded from consideration were any teeth exhibiting symptoms of fracture, severe decay, or blockage in the root canal. After cleaning, the extracted teeth were kept in saline until needed. A diamond disc was used to flatten the tooth's occlusal surface and standardise its length to 16 mm (Antony *et al.*, 2020). Included in the study were teeth without pulp stones, calcification, dentinal cracks, resorption, or two canals. A single skilled operator carried out the biomechanical preparation. Access was obtained using a carbide bur, the working length was determined using a 15 K file, and the working length was maintained 1 mm short of the radiography apex. SX rotary files are used for coronal enlargement, Profit S3 rotary files are used for apical enlargement up to size PF2, and 6% taper is employed by manufacturer instructions until the working length, using the coronal part as a stable reference point.

The interior diameter was then standardized using a slow-speed handpiece (NSK, Tokyo, Japan) and a Gates Glidden drill no. 3 (produced by Mani Inc., Tochigi-ken, Japan). 3 percent NaOCl was used to soak the teeth. The teeth were then autoclaved for 20 minutes at 121°C after being submerged in distilled water for 10 minutes to eliminate any remaining chemical traces. (Moura *et al.*, 2004)

After that, an *Enterococcus faecalis* strain – used as a test organism for this study—was utilized to contaminate the specimens. The Cultures were grown in brain infusion (BHI) broth provided by Becton Dickinson and Company, Sparks, MD, suspended in 5 ml of TS medium, and incubated at 37°C for 4 h (figure 1). The turbidity met the McFarland threshold of 0.5. One millilitre of tryptone soy broth was placed in each of the 50 μL pre-sterilized microcentrifuge tubes together with the *Enterococcus faecalis* inoculums. After a full day (Figure 2), The samples were transferred to fresh broth containing E. faecalis and stored in a laminar flow environment (fresh air, Mumbai, India). To ensure culture quality, 5 μL of sap from dentin cells incubated in TSB was subcultured on tryptone soy agar plates. *Enterococcus faecalis* was cultured with the materials for 21 days at 37 degrees Celsius. (Moura *et al.*, 2004)

After 21-days the specimens were incubated for the full length, the contaminated broth was eliminated with a 5 mL sterile water wash. In accordance with the irrigation methodology, the collected samples are splitted into three categories. using a computer-generated randomization method (www.random.org). as:

Group 1: Double-side vented needle (30 G) (n=15)

Group 2: Conventional needle irrigation (n=15)

Group 3: Endoactivator (n=15)

A single operator performed the entire irrigation process and was blinded until the study's conclusion. Once the operator had received the appropriate specimens, the protocols were explained to him. Protocols varied depending on the groups.

Group I: Double-side vented needle (n=15)

20 mL of 3% NaOCl (Acquafarma; Niteroi, RJ, Brazil) was used for irrigation, and 5 mL of distilled water was used for a last rinse. Only a 30-gauge double-vented needle coupled to a 5-mL water syringe barrel was employed by this group.

Group II: Conventional needle irrigation

20 mL of 3% NaOCl (Acquafarma; Niteroi, RJ, Brazil) was used for irrigation, and 5 mL of distilled water was used for a last rinse. Irrigation was performed using 24 gauge conventional needle irrigation.

Group III: Endoactivator

It comes with 5 mL disposable plastic syringes, and 30 gauge side vented needles for Irrigation. One millimeter away from the working length, an EA handpiece with a red (25/04) tip set at 10,000 cycles per minute was inserted. After 10 mL of 3% NaOCl (Acquafarma; Niteroi, RJ, Brazil) was injected, the canal was stirred for one minute. Automatic irrigation equipment was used to irrigate 20 ml of 3% NaOCl.

Dentin fragments from the apical third of the canal were collected using Gates Glidden 1 following the irrigation process. This was followed by GG 2, 3, 4, and 5. The procedure involved adding 1 mL of sterile BHI to the samples for suspension, then creating ten-fold dilutions and spreading the suspensions onto BHI agar substrate in 0.1 mL aliquots. After 48 hours of incubation at 37°C, the colony-forming units (CFUs) were enumerated. The ground root end's weight was used to calculate CFU/mg. Similar techniques were employed to sample and cultivate specimens from the control teeth. Verification was conducted to confirm the sterility of the negative controls and the purity of the positive cultures.

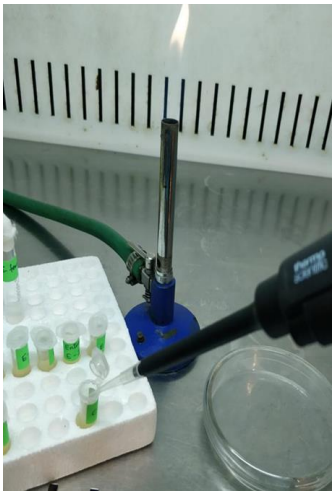
Statistical analysis:

The gathered data underwent analysis using IBM SPSS Statistics version 23.0 for Windows (IBM Corp., Armonk, NY, USA). To determine any significant differences between the groups under consideration, the Kruskal-Wallis test was used.

Table 1: Table Depicting the Statistical Difference in Number of *Enterococcus faecalis* CFU Counts in Various Groups Compared.

Groups	N	Mean	Std. Deviation	Minimum	Maximum	P-Value
Group I	15	165.21	45.17	130	180	0.001

Group II	15	350.32	52.02	300	372	0.001
Group III	15	143.28	41.10	110	150	0.001



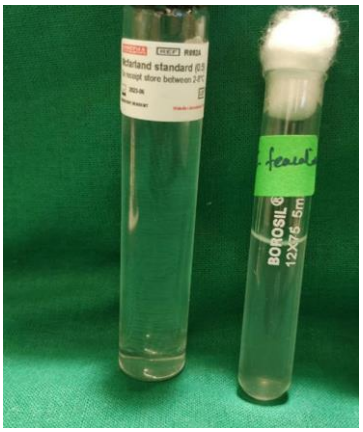


Figure 1: *Enterococcus faecalis* grown on tryptone soya agar (TSB), suspended in 5 mL of TS broth

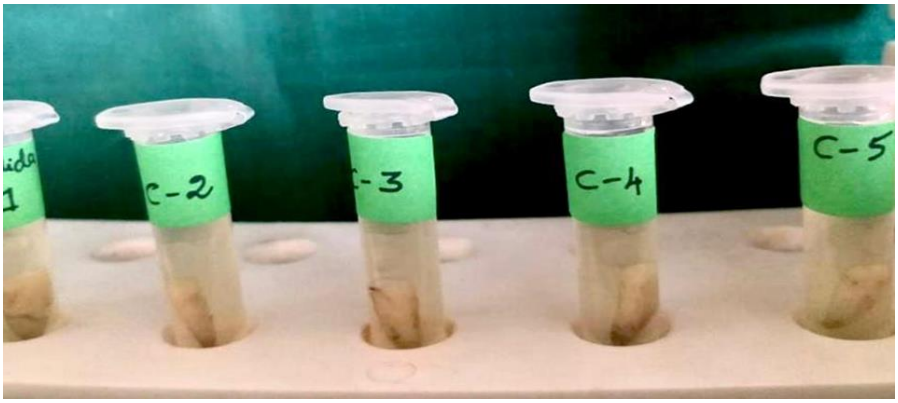


Figure 2: Teeth were transferred into the fresh broth containing E. faecalis

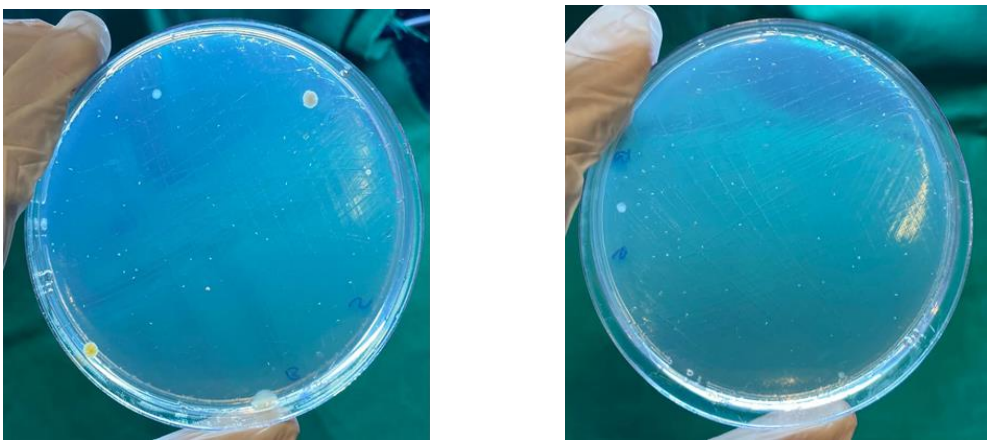


Figure 3: Colony forming units (CFU) were seen on the petri dish and calculated
The Figure 3 shows the Colony forming units (CFU) were seen on the petri dish and calculated

Results:

There was a statistically significant variance ($p < 0.05$) in the reduction of *Enterococcus faecalis* among the experimental groups. Groups I and III showed a significant difference ($P<0.05$). In Table 1, CFU counts from various groups were compared and tabulated. From the very beginning of the experiment, *Enterococcus faecalis* contamination of all specimens was confirmed. The sample's CFU/ml counts showed statistically significant differences between three groups. On the petri dish, a colony was forming, and its number was calculated.

DISCUSSION:

The EndoActivator, developed by Ruddle, Sharp, and Machtou, uses sonic energy to vigorously agitate an irrigant to disturb biofilm and the smear layer through a hydrodynamic phenomenon that causes cavitation and acoustic streaming deep cleaning and disinfection (Susila and Minu, 2019). Siu and Baumgartner (2010) reported that EndoVac was able to accomplish superior cleaning at the apical third with only 150 seconds of exposure time. The effectiveness of debridement was evaluated using ex vivo histological staining. Activation with EndoVac was more effective than traditional irrigation, particularly in the apical third. (Siu and Baumgartner, 2010)

The higher efficiency and reduced cytotoxicity of the 2.5% NaOCl made it the preferred solution. Yet, it was shown that 6% NaOCl was the most effective disinfection for a 3-week-old Enterococcus faecalis biofilm. (Cai *et al.*, 2023) The studies tested the efficacy of different incubation times—two weeks, three weeks, and four weeks—for *Enterococcus faecalis* disinfection techniques. According to research by other researchers, the *Enterococcus faecalis* biofilm did not fully develop itself for three weeks. [1,15]Cleaning untouched walls and places inaccessible to rotary instruments requires efficient irrigation. When compared to manual irrigation with a syringe and needle, additional irrigant activation may reduce the debris extrusion and enhance the elimination of the smear layer from the canal lumen.

The conventional needle irrigation method, commonly utilized, entails the replenishment and exchange of fluid within a narrow range just above the tip of the irrigation needle, typically around 1-1.5 mm apical. In this method, an irrigation needle is connected to a syringe. The vapour lock

effect is a problematic element that hampers the effectiveness of the syringe-needle irrigation method, When air is drawn into the apical region of the root canal, it prevents water from reaching the apical region of the root canal.(Generali *et al.,* 2017) (Abu Hasna *et al.,* 2021) The EndoActivator gadget from Dentsply Sirona in Ballaigues, Switzerland, is a wireless, transportable handpiece with an extremely flexible polymer tip that fluctuates between 1 and 10 kHz. The tip's design permits both forceful and safe agitation of the intracanal fluid. When brief vertical strokes are combined with horizontal agitation at the tip, they create a powerful hydrodynamic effect within the root canal. Cleaning is more successful because it improves irrigant circulation, transverse permeation, and flow into the root canal's inaccessible areas. (Parikh *et al.,* 2019) PUI was less effective in removing the smear layer from the endodontic walls from the apex to the crown. (Machado *et al.,* 2021) (Mancini *et al.,* 2013) Hockett et al. conducted an in vitro study in which they collected fluid and dentin fragments from the interior of canals following shaping and cleaning. Their research aimed to compare the percentage *Enterococcus faecalis* growth in root canals, when the EndoVac technology or needle irrigation was used. According to their findings, positive pressure needle irrigation did not consistently provide the same level of microbial control as apical negative pressure administration of irrigants via the EndoVac system. This implies that the EndoVac system could potentially be more effective in managing microbial contamination during root canal procedures compared to traditional needle irrigation methods.(Miller and Baumgartner, 2010)

Future research should also focus on investigating the effectiveness of root canal disinfection against microbial clusters and biofilms.

CONCLUSION:

According to the findings of this study, it can be concluded that the different irrigation protocols significantly reduce bacterial efficiency and increase clinical efficacy. Within the limitations of the study, the Endoactivator and double-side vented needle were better in bacterial reduction compared to conventional needle irrigation.

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