

# Antibacterial Efficacy of Calcium Hydroxide with Iodoform versus Calcium Hydroxide with Camphorated Paramonochlorophenol as Intrachannel Pastes on an *Enterococcus faecalis* Biofilm: A Comparative *In Vitro* Study

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**ABSTRACT** **Objective:** The objective of this study was to assess *in vitro* the antibacterial efficacy of Ca(OH)<sub>2</sub> with iodoform versus Ca(OH)<sub>2</sub> with camphorated paramonochlorophenol as intrachannel pastes on an *Enterococcus faecalis* biofilm. **Materials and Methods:** The diffusion method was used in wells. The strain used was *E. faecalis* ATCC 29212. Bile esculin agar was inoculated into 60-well plates of 5 mm in diameter. Three groups were formed: Group 1: Calen PMCC (Ca(OH)<sub>2</sub> + camphor paramonochlorophenol); Group 2: Metapex (Ca(OH)<sub>2</sub> + iodoform); and Group 3: camphor paramonochlorophenol inoculated with *E. faecalis* as a positive control. The plates were then incubated at 37°C for 24 h. Bacterial inhibition halos were read. **Results:** Group 1 presented the highest antimicrobial efficacy with a mean of 16.2 ± 0.6 mm, whereas Group 2 only had an antimicrobial effect of 9.7 ± 1.3 mm. Finally, Group 3 only exposed to the positive control (camphor paramonochlorophenol) showed an effect of 14.6 ± 1.0 mm. The inferential analysis showed statistically significant differences between the antimicrobial effect of the three groups ( $P = 0.001$ ). **Conclusion:** Ca(OH)<sub>2</sub> paste with camphor paramonochlorophenol (Calen PMCC) has a greater antibacterial action on *E. faecalis*. The iodoform-associated Ca(OH)<sub>2</sub> paste (Metapex) showed significantly lower antibacterial action against *E. faecalis* ( $P < 0.05$ ).

**KEYWORDS:** Antibacterial efficacy, *Enterococcus faecalis*, *in vitro* study, intraconduction medication

## INTRODUCTION

The main goal of endodontic treatment is to eliminate the microorganisms that are in the root canal and are the cause of inflammation and periapical infection, and thus prevent reinfection. Root canal flora not responding to endodontic treatment is made up of a limited number of predominantly gram-positive microbial species. According to several studies, the predominant microorganisms in teeth with apical periodontitis are *Streptococcus mutans*, *Enterococcus*, *Lactobacillus*, and *Candida*, which can apparently survive chemomechanical treatment of the root canal.<sup>[1-3]</sup>

One of these microorganisms, *Enterococcus faecalis*, has great capacity for adaptation and tolerance to adverse environmental conditions, making eradication difficult. The action of different antimicrobials used to eliminate infectious agents in the root canal has been studied. However, the resident flora in pieces undergoing root canal treatment, which have been sealed and present

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persistent periapical disease, poses a constant challenge to maintain the balance of host–pathogen interaction in an effort to maintain a physiological state without signs and symptoms and a favorable treatment evolution and more predictable results.<sup>[3,4]</sup>

Technical and scientific advances have made root canal therapy a reliable procedure with favorable prognoses. The goal is to completely eradicate these biological agents and their by-products. This can be achieved through the use of different techniques (instrumentation, endodontic irrigants, intracanal medication, and obturation). However, despite the advances in treatment techniques, some patients may present exacerbations between appointments or exacerbations following completed endodontic treatments. Numerous studies have shown the importance of bacterial elimination for successful root canal treatment. However, total elimination of microorganisms from the root canal can be very difficult or almost impossible.<sup>[2-4]</sup>

Various factors such as the use of inadequate mechanical instrumentation techniques, insufficient irrigation, not administering intraconduction medication between appointments, and poor provisional fillings have been described as the cause of persistent infection after treatment. Nonetheless, some epidemiological studies have reported cases in which despite special care of these factors, recurrent infections occurred, suggesting the presence of other determining factors not controlled by the operator, such as microbiological factors.<sup>[5-7]</sup>

Several studies have evaluated the use of natural products as a new antimicrobial alternative,<sup>[8-11]</sup> and have described the importance of the application of substances such as intraconduction medication in pieces with asymptomatic apical periodontitis to eliminate existing bacteria, and also to achieve successful duct treatment.

Therefore, the aim of this study was to compare the *in vitro* antibacterial efficacy of  $\text{Ca}(\text{OH})_2$  plus iodoform versus  $\text{Ca}(\text{OH})_2$  with camphorated paramonochlorophenol as intrachannel pastes on an *E. faecalis* biofilm.

## MATERIALS AND METHODS

This research was carried out in the Microbiology Laboratory and in the Graduate Unit of the Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos (UNMSM) in Lima, Peru.

### STUDY DESIGN AND SAMPLE SIZE CALCULATION

This was a prospective, experimental, *in vitro* study, and it had no relevant ethical implications. The analysis unit consisted of the wells inoculated with *E. faecalis*

present in refractory chronic apical periodontitis. The sample size was calculated with the mean comparison formula using an  $\alpha = 0.05$  and a  $\beta = 0.80$  with the Stata, version 15.0, statistical software. The sample corresponded to 60 wells inoculated with the *E. faecalis* ATCC (American Type Culture Collection) 29212 biofilm.

### ALLOCATION

The following groups were formed to evaluate antimicrobial efficacy:

- Group 1: Calen PMCC ( $\text{Ca}(\text{OH})_2$  + camphor paramonochlorophenol) inoculated with *E. faecalis* ATCC 29212 ( $n = 20$  wells)
- Group 2: Metapex ( $\text{Ca}(\text{OH})_2$  + iodoform) inoculated with *E. faecalis* ATCC 29212 ( $n = 20$  wells)
- Group 3: Camphor paramonochlorophenol inoculated with *E. faecalis* ATCC 29212 ( $n = 20$  wells)

### REACTIVATION OF THE STRAIN

The strain was reactivated according to the protocol of MicroBiologics Laboratory (St. Cloud, Minnesota). The culture medium used was bile esculin agar (BEA) (Laboratorios Britania SA., Buenos Aires, Argentina), incubated at 37°C for 72h under aerobic conditions [Figure 1].

### PREPARATION OF THE CULTIVATION MEDIA

The BEA culture medium was prepared by mixing 40g of the dehydrated product in 1L of distilled water. The mixture was then subjected to heat until complete dissolution, and was then autoclaved for 15min at 121°C. After sterilization, this dissolution was kept at a temperature of 50°C, and then approximately 20mL was added to each of the disposable plastic petri dishes [Figure 2].



Figure 1: Sowing the *Enterococcus faecalis* strain

### BACTERIAL SEEDING

Three or four colonies of the reactivated strain were extracted with a seeding loop and inoculated into 5 mL of physiological serum to stabilize the inoculum at 0.5 of the Mac Farland Scale ( $1.5 \times 10^8$  colony forming units [CFU/mL]). Then 100  $\mu$ L was taken with sterile tips and an automatic pipette, placed on the plate and then spread with a sterile swab over the BEA in the petri dishes. The diffusion test was used for measuring the halos of the three study groups as follows: Group 1: Calen PMCC:  $\text{Ca}(\text{OH})_2$  pastes with 2.7 g of  $\text{Ca}(\text{OH})_2$  paste with camphor paramonochlorophenol and 2.2 g of glycerin (SS 33 White Artigos Dentários, Rio de Janeiro, Brazil); Group 2: Metapex:  $\text{Ca}(\text{OH})_2$  associated with iodoform with silicone oil (Meta-Biomed, Chungcheongbuk-do, Korea); and Group 3: *E. faecalis* ATCC 29212 in Kwik-Stik from MicroBiologics.

### ANTIBACTERIAL EFFICACY

Six 5 mm in diameter wells were prepared on each plate using a sterile glass rod, making a total of 60 wells. In each well, the pastes to be evaluated were inoculated in duplicate up to the level of the culture medium, using a No. 27 carpule syringe for the Calen PMCC, and with a pipette placed in the syringe containing the product for the Metapex. In addition, 40  $\mu$ L of camphorated paramonochlorophenol was added for the positive control, and three drops of glycerin for the negative control. The plates were then placed in the Fravril Bionet S.A. (Lima, Peru) incubator at 37°C. After 24h, the bacterial inhibition zones expressed in mm of diameter were read with a digital caliper 12"/0.01 mm  $\times$  0.0001" Mitutoyo 500-193, taking into account the average of the diameters formed around each substance

evaluated at 24h, and the results were recorded in the data collection table [Figure 3].

### STATISTICAL ANALYSIS

To carry out the descriptive analysis, the measures of central tendency and dispersion (mean, standard deviation, minimum value, and maximum value) of the antimicrobial efficacy of the experimental groups evaluated were obtained. The Shapiro–Wilk test was used to assess normality, showing that all groups presented a normal distribution. Finally, to perform the inferential analysis, the analysis of variance (ANOVA) test and Tukey *post hoc* test were used. All the statistical analyses were performed with Stata, version 15.0, statistical software, and the level of significance was established at  $P < 0.05$ .

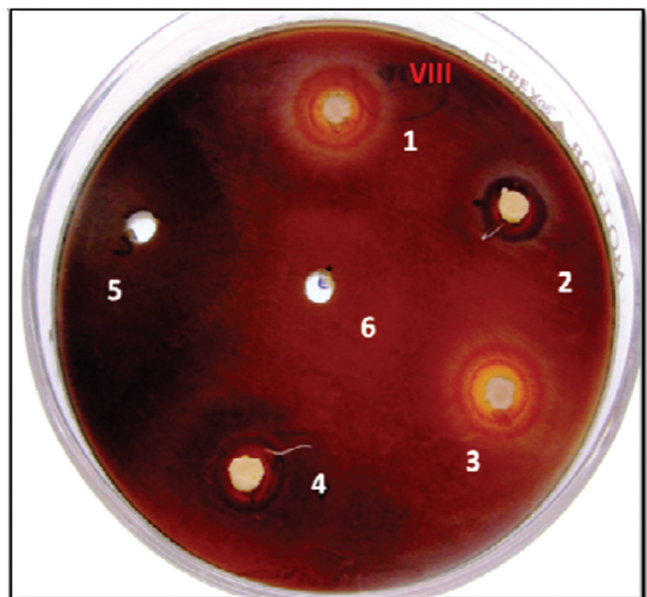
### RESULTS

Group 1 (Calen PMCC group) presented the highest antimicrobial efficacy with a mean of  $16.2 \pm 0.6$  mm, whereas Group 2 (Metapex group) only had an antimicrobial effect of  $9.7 \pm 1.3$  mm, and Group 3 that was only exposed to the positive control (camphor paramonochlorophenol) showed an effect of  $14.6 \pm 1.0$  mm. The inferential analysis showed statistically significant differences among the antimicrobial effects of the three groups ( $P = 0.001$ ) [Table 1, Graph 1].

*Post hoc* analysis of the *in vitro* comparison of the antimicrobial efficacy of the modified  $\text{Ca}(\text{OH})_2$  endodontic pastes found statistically significant differences among the three groups ( $P = 0.001$ ) [Table 2].



**Figure 2:** Petri dishes incubated for evaluation of antimicrobial efficacy



**Figure 3:** Measurement of the halos of the experimental groups in cultures of *Enterococcus faecalis*

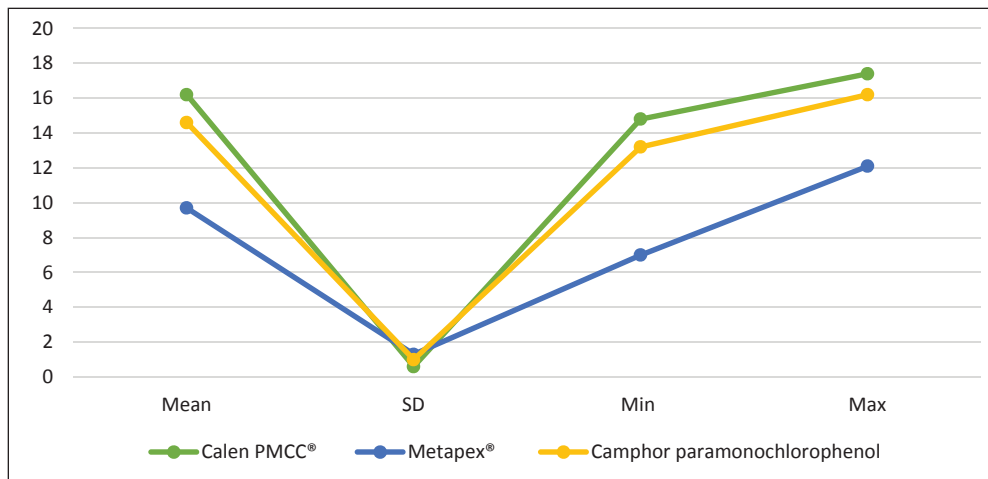
**Table 1: In vitro evaluation of the antimicrobial efficacy of modified calcium hydroxide endodontic pastes**

Groups assessed	Mean	SD	Min.	Max.	P*	P**
Calen PMCC	16.2	0.6	14.8	17.4		
Metapex	9.7	1.3	7.0	12.1	0.05	0.001
Camphor paramonochlorophenol	14.6	1.0	13.2	16.2		

Calen PMCC = (Ca(OH)<sub>2</sub> + Camphor paramonochlorophenol), Metapex = (Ca(OH)<sub>2</sub> + Iodoform), camphor paramonochlorophenol = positive control, SD = standard deviation, Min = minimum, Max = maximum

\*Shapiro–Wilk normality test, \*\*ANOVA test; Significance level at P < 0.05

All inhibition halos were measured in millimeter



**Graph 1:** Descriptive analysis of the antimicrobial efficacy of modified endodontic pastes based on calcium hydroxide

**Table 2: Post hoc analysis of the in vitro comparison of the antimicrobial efficacy of modified calcium hydroxide endodontic pastes**

Groups assessed	Mean differences	Confidence intervals	P*
Calen PMCC	Metapex	5.6 7.3	0.001
	Camphor paramonochlorophenol	0.5 2.5	0.001
Metapex	Calen PMCC	-7.3 -5.6	0.001
	Camphor paramonochlorophenol	-5.9 -3.9	0.001
Camphor paramonochlorophenol	Calen PMCC	-2.5 -0.5	0.001
	Metapex	3.9 5.9	0.001

Calen PMCC = (Ca(OH)<sub>2</sub> + camphor paramonochlorophenol), Metapex = (Ca(OH)<sub>2</sub> + Iodoform), camphor paramonochlorophenol = positive control

\*Tukey test, significance level P < 0.05

All inhibition halos were measured in millimeter

**DISCUSSION**

As bacteria play a major role in the pathogenesis of pulpal and periradicular lesions, cleaning and disinfection of the root canal are vital to minimize the risk of bacterial growth. The persistence of resistant microorganisms in pieces with previous endodontic treatment and asymptomatic apical periodontitis is one of the main challenges that the endodontists face. Bacteria such as *E. faecalis* are the most difficult to eradicate and produce greater problems after treatment.<sup>[12-15]</sup>

*E. faecalis* is frequently found in cultures of obturated root canals showing signs of asymptomatic apical periodontitis. Although chemomechanical debridement removes a substantial amount of bacteria, some microorganisms remain in the dentinal tubules, lateral canals, and other irregularities, favoring reinfection of the endodontic space. Consequently, during the treatment of root canals, it is advisable to implement a temporary medicine, which is in direct contact with the walls of the root canal, to minimize the risk of bacterial growth. Intraconducting drugs are used to eliminate

bacteria from the root canal, act as a physicochemical barrier, and decrease the nutrients necessary for bacterial proliferation.  $\text{Ca}(\text{OH})_2$  has long been the medicine of choice, as it eliminates residual microorganisms by having a high pH of 12–12.8, and also prevents periapical filtration of exudate into the endodontic system.<sup>[16-18]</sup>

Taking into account the frequent use of intraconduction medication by dental professionals, it is of interest to know the antimicrobial action exerted by these drugs against resistant bacteria. Several studies have reported resistance of *E. faecalis* to the antibacterial action of  $\text{Ca}(\text{OH})_2$  due to its great ability to adapt to alkaline mediums of up to a pH of 11.<sup>[2,4,6]</sup>

Therefore, the search for better alternatives has led to the preparation of various  $\text{Ca}(\text{OH})_2$  formulations using different vehicles and new antimicrobial agents such as propylene glycol, iodoform, silicone, and paramonochlorophenol. To date, there are few studies comparing the antibacterial action of  $\text{Ca}(\text{OH})_2$  paste with iodoform against *E. faecalis*.<sup>[12,13,16,17]</sup> The materials evaluated in this study were selected because the commercial formulation of  $\text{Ca}(\text{OH})_2$  paste with iodoform (Metapex) and that of  $\text{Ca}(\text{OH})_2$  paste with camphor paramonochlorophenol (Calen PMCC) are accessible in the Peruvian market.

According to a study by Sáez *et al.*,<sup>[19]</sup> changes in pH and diffusion of calcium ions through root dentin from  $\text{Ca}(\text{OH})_2$  pastes and aggregate of mineral trioxide (MTA) led to the release of calcium ions in all the groups.  $\text{Ca}(\text{OH})_2$  paste with distilled water had the highest calcium ion value at 60 days ( $P \leq 0.01$ ). There was a positive correlation between calcium and pH values. Conversely, Zancan *et al.*<sup>[20]</sup> evaluated the release of calcium, the solubility, and the antimicrobial action of  $\text{Ca}(\text{OH})_2$ , Calen (SS White Artigos Dentáriosil), Calen camphor monochlorophenol, and  $\text{Ca}(\text{OH})_2$  plus chlorhexidine pastes against biofilms. Seven days of contact were found to be insufficient for the  $\text{Ca}(\text{OH})_2$  pastes plus the aggregates to kill the bacteria in the biofilms studied. However, chlorhexidine added to the  $\text{Ca}(\text{OH})_2$  favored greater effectiveness against the aforementioned bacterial biofilms.

On the contrary, Campanella *et al.*<sup>[12]</sup> investigated the efficacy of endodontic sealants and endodontic medications Aureoseal, MTA,  $\text{Ca}(\text{OH})_2$ , and iodoformed paste against *E. faecalis* in 36 plates inoculated with the experimental suspensions. Similar to this study, these authors also used the agar diffusion method to investigate the antimicrobial activity of root canal sealants. The diameters of the microbial inhibition zones were measured in millimeters around the plate. They showed that the antimicrobial activity

of Aureoseal was superior to that of MTA, iodoformed paste, and  $\text{Ca}(\text{OH})_2$  in the microorganism analyzed. Similar to our study, this study confirmed the resistance of *E. faecalis* to endodontic sealants. Aureoseal and  $\text{Ca}(\text{OH})_2$  showed the best results in the inhibition test, suggesting that they may be useful in clinical cases.

Another study that coincided with the results of our study was that by Saha *et al.*<sup>[14]</sup> who described that microbes are considered the main etiological agents in endodontic diseases and may be reduced by root canal debridement and antibacterial filler materials. Previously, one of the factors determining the success of an endodontic treatment was to seal the root canals with materials that have a powerful bactericidal effect. However, due to the cytotoxic reactions of the sealants and their inability to completely remove bacteria from the dentinal tubules, there is now a trend to using natural plant extracts.<sup>[8-11]</sup> A sealant based on zinc oxide and eugenol with herbal extracts produced larger inhibitory zones followed, in descending order, by a resin-based sealer, and  $\text{Ca}(\text{OH})_2$  along with the three herbal extracts.

The main limitation of this study was that as it was a purely experimental *in vitro* study, other factors that may occur in clinical practice that may enhance the antimicrobial activity of the endodontic pastes evaluated could not be controlled. On the contrary, another limitation was that we only evaluated the antibacterial efficacy on the different materials against the main bacteria of endodontic infections (*E. faecalis*), although it is known that other endodontic bacteria with infection potential may exist.

Finally, future studies using mixed oral microflora obtained from ducts diagnosed with asymptomatic apical periodontitis are needed to guarantee applicability to clinical practice. In addition, studies in human dentin models should be performed to verify the action of endodontic pastes on the dentinal tubules, as there are few studies on permanent teeth. Indeed, only clinical studies with Metapex in deciduous dentition diagnosed with pulp necrosis have mainly been carried out. Although a high percentage of success has been achieved with endodontic pastes, only few studies are available in permanent dentition.

## CONCLUSION

In summary,  $\text{Ca}(\text{OH})_2$  paste with camphor paramonochlorophenol (Calen PMCC) has greater antibacterial action on *E. faecalis* compared  $\text{Ca}(\text{OH})_2$  + iodoform (Metapex) and camphor paramonochlorophenol, with the second paste presenting significantly lower antibacterial action against *E. faecalis* ( $P < 0.05$ ).

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Nil.

**CONFLICTS OF INTEREST**

None to declare.

**AUTHOR CONTRIBUTIONS**

Study conception (MJ, DSM), data collection (MJ, DSM), data acquisition and analysis (RW, FMT, GA), data interpretation (RW, DSM, FMT), manuscript writing (FMT, DSM, GA, RW).

**ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT**

This project is exempted from ethical approval due to it was an experimental in vitro study. All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki.

**PATIENT DECLARATION OF CONSENT**

Not Applicable.

**DATA AVAILABILITY STATEMENT**

The data that support the study results are available from the author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@ucientifica.edu.pe) on request.

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