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A comparative evaluation of antibacterial efficacy of 'calcium hydroxide plus points', 'activ points' and 'combi points' against *Enterococcus faecalisin* endodontic therapy: an *in vitro* analysis

Abstract

The success of endodontic therapy relies on complete eradication of microorganisms from the root canal system. However, there are some resistant strains such as *Enterococcus faecalis* which persist in the root canals, leading to endodontic failures. Various antimicrobial agents have been tested time to time for their efficiency against this pathogen. The purpose of this study was to compare the antimicrobial efficacy of calcium hydroxide releasing 'Calcium hydroxide plus points'; chlorhexidine releasing 'Activ points', chlorhexidine and calcium hydroxide releasing 'Combi points' against *Enterococcus faecalis*. By measuring the zone of inhibition, it was concluded that 'Calcium hydroxide plus points were not effective, while both Activ points and Combi points showed results significantly better than Calcium hydroxide plus points. The results indicate the calcium hydroxide alone may not be effective when expecting *Enterococcus faecalis* in particular endodontic cases and must be replaced or adjuncted by the use of chlorhexidine.

Keywords: activ points, calcium hydroxide, chlorhexidine, combi points, *Enterococcus faecali*, mono infection, bactericidal properties, formocresol

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Introduction

Bacteria and their products play a primary etiological role in the initiation and perpetuation of pulpo-periapical pathosis. The biological objective of root canal treatment is eradication of bacterial load from the root canal system. Despite the fact that all measures are being taken to achieve this goal, failures are still being reported.[1] The ability of certain microbes to form biofilms is one of the key factors in their pathogenesis of endodontic infection. These biofilms not only adhere strongly to the dentin but also penetrate deep into the dentinal tubules.[2] Also, there are certain bacteria which are, in general, resistant to many chemical reagents used in endodontic therapy. Amongst these resistant bacteria, *Enterococcus faecalis* has been found to be a major isolate.[3] Its prevalence in failure root canal cases ranges from 24-77%,[4] probably owing to its ability to establish mono infection. It can also get converted into viable but non-cultivable state and can survive low pH, high salinity and high temperatures. It has also been observed that it encompasses Gene encoded antibiotic resistance. A number of anti-microbial irritants and medicaments have been tried in endodontics since years, expecting to completely eliminate this bacteria from the root canals system such as calcium hydroxide, formo-cresol, camphorated para-mono-chlorophenol, chlorhexidine, etc.[5–8] Calcium hydroxide has bactericidal properties since it inhibits bacterial enzymes by means of hydroxyl ions of the bacteria's cytoplasmic membrane, generating the antibacterial effect.[9] Its high pH (around 12.5) has a damaging effect on cell membranes and protein structure.[10]

Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent that has been used as an effective intra canal medicament. It is a synthetic cationic bis-guanide, which consists of two symmetric 4-cholorophenyl rings and two biguanide groups, connected by a central hexamethylene chain,[11] its effectiveness is because of altering the microbial cells osmotic equilibrium due to the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls. CHX molecule penetrates into the bacteria due to increase in permeability of the cell wall.[12] It is a well-established fact that chlorhexidine alone or a combination of chlorhexidine and calcium hydroxide has very high efficiency against *E. faecalis*.[13] The evaluation of chlorhexidine as intracranial medicaments in the form of sustained releasing points (Activ points) alone or in combination with calcium hydroxide (Combi Points) has already been done.[13,14] The present *in vitro* study was undertaken to evaluate the antimicrobial efficacy of calcium hydroxide releasing 'Calcium hydroxide plus points' as compared 'Activ Points' and 'Combi points' against *E. faecalis* so as to analyze, if at all the sustained release of calcium hydroxide from the points has any better advantage.

Materials and methods

The standard protocol of maintaining *E. faecalis* (ATCC 47077) on agar plates was use.[13] Thirty Mc Konkey agar plates were inoculated with the bacteria. These agar plates were divided into three groups, depending on the test specimen used. 'Calcium Hydroxide plus points' (Roeko, Germany) were designated as Group I, chlorhexidine releasing 'Activ points' (Roeko, Germany) designated as Group II; and both calcium hydroxide and chlorhexidine releasing 'Combi points'(Roeko, Germany) were designated as Group III. The two test agents of same group were placed in each plate at a distance (Figure 1). Then the McKonkey agar plates were incubated for 24hours at 37°C aerobically. Zones of inhibition indicating efficacy of the drug were measured after 48 hours using digital vernier caliper (Figure 2).

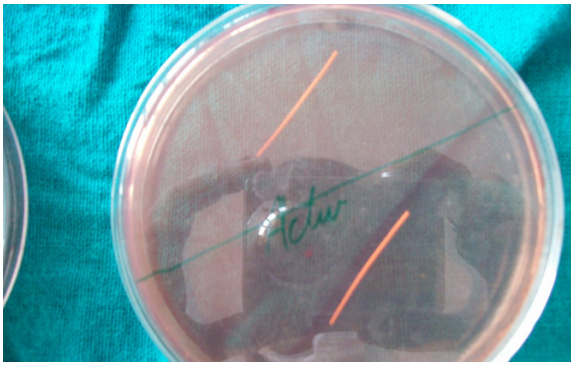

Figure 1 Test specimens placed in Mc Konkey agar plate.

Figure 2 Zone of inhibition measured by digital vernier caliper.

Observations and results

Table 1 represents the zones of inhibition measured after 48 hours. While, no zone of inhibition was seen in samples for Group I (Figure 3). Zone of inhibition was seen in all the samples of Group II (Figure 4) and III (Figure 5). The mean zone of inhibition of each group was calculated and subjected to statistical analysis (paired T-test) for the mean zones of inhibition between the different groups. The significance value was set at $p<0.05$=significant. When compared, a statistically significant difference was found between Group I and II, with latter performing better than former ($p<0.05$=significant). Also, when Group III was compared to Group I, it performed and this was found to be statistically significant ($p<0.05$=significant). When group III was compared to group II, it was found that no significant difference was observed between the two groups.

Table 1 Zone of inhibition (in mm) measured for all the three groups after 48 hours

S. No	Group I	Group II	Group III
1	0	6.17	6.1
2	0	6.96	6.58
3	0	5.87	4.75
4	0	5.71	4.9
5	0	6.67	6.55
6	0	6.86	6.11
7	0	6.29	5.9
8	0	6.7	6.5
9	0	5.19	4.74
10	0	5.3	6
11	0	6.25	5.9
12	0	6.4	6.26
13	0	6.28	5.56
14	0	6.6	6
15	0	6.88	6.5
16	0	6.12	6
17	0	6.5	6.25
18	0	7.18	7.19
19	0	7.9	7.72
20	0	7.26	7.1

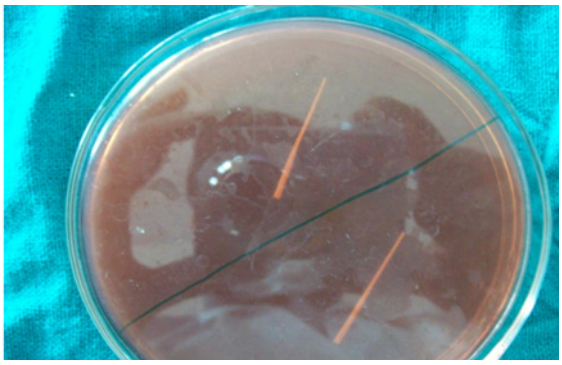

Figure 3 No zone of inhibition seen in Group I sample.

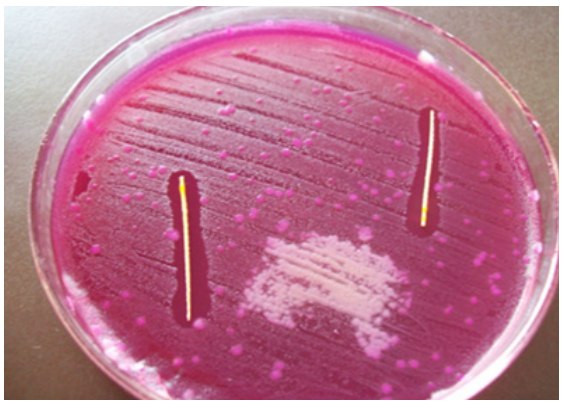

Figure 4 Zone of inhibition seen in Group II sample.

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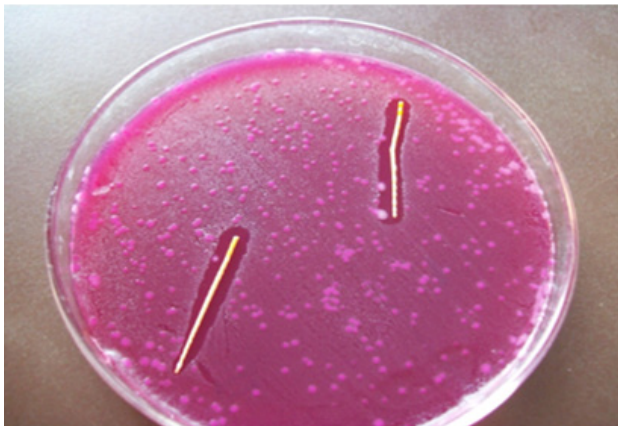

Figure 5 Zone of inhibition seen in Group III sample.

Discussion

For the successful performance of the endodontic therapy, both the mechanical and biological principles of root canal preparation must be followed. While the mechanical principles are often strictly adhered to, the biological once are, at times, overlooked. It is the presence of these remnant bacteria in the root canal system, which is commonly associated with the endodontic failure. Complex microbial flora consisting of cocci, rods, spirochetes, filaments and sometimes fungi are present in root canals of infected teeth.[15] One of the most resistant microbes found in root canal system is *E faecalis*. It is a Gram-positive, catalase negative, fermentative and non-sporing facultative anaerobic coccus. Its cells are ovoid with diameter ranging from 0.5 to 1µm. *Enterococci* possess several virulence factors that assist adherence to host cells and extracellular matrix and assist in tissue invasion. These factors include: aggregation substance; enterococcal surface proteins such as gelatinase; cytolysin toxin; extracellular superoxide production; capsular polysaccharides and antibiotic resistance determinant.[16,17] One significant feature contributing to pathogenic potential of *E. faecalis* is its ability to adhere and infiltrate into dentine.

The analysis of the fact that which antimicrobial agents are effective against *E. faecalis* is of utmost importance. The use of calcium hydroxide in eliminaition of *E. faecalis* has been tested previously and results have not been very favorable. It has been postulated that *E. faecalis* is resistant to calcium hydroxide.[18,19] The results obtained in our study match with these previous tests. Siren et al.,[20] in their study confirmed the effectiveness of chlorhexidine as an intracanal medicament in cases where *E. faecalis* was suspected. Similar conclusion has also been reported by Lui et al.,[21] and Singh et al.,[22] Also, there have been studies earlier which suggest that combined use of chlorhexidine and calcium hydroxide is also effective against *E. faecalis*.[13,23]

No statistically significant difference was observed in zone of inhibition between Activ points and Combi points in this study. The use of agar diffusion test for measuring the efficiency of antibacterial agents has always been doubted since the simulation with actual clinical condition is very less. But this procedure is one of the most easily available methods for the researchers all across the globe.[24] Therefore within the limitations of this study, it can be concluded that the use of calcium hydroxide alone to combat *E. faecalis* in endodontic infections may be insufficient and thus use of chlorhexidine in replacement or as an adjunct to it may be a better option.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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