Original Research

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF TRIPLE ANTIBIOTIC PASTE AND MODIFIED DOUBLE ANTIBIOTIC PASTE USING DIFFERENT VEHICLES AGAINST ENTEROCOCCUS FAECALIS

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Abstract:

Microorganisms and their products are considered as the etiological agents of pulp necrosis and periradicular lesions. Many studies have reported that E. faecalis is cultured in higher frequency from root canals of teeth with failed endodontic treatment. To ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required. So, the aim of the present study was to evaluate the antimicrobial activity of triple antibiotic and modified double antibiotic paste with different vehicles against E.faecalis. The antimicrobial effect was evaluated by the direct contact with agar diffusion method. The freshly prepared medicaments were placed in wells on agar plates inoculated with different microorganisms. All plates were incubated and the diameter of bacterial inhibition zones on agar plate was measured in millimeters at 24, 48 and 72 hours at 37°C and 100% humidity. The results showed that the Modified Double antibiotic paste irrespective of vehicle was more effective than triple antibiotic paste against E.faecalis.

Key words : Triple antibiotic paste , Modified double antibiotic paste, Chitosan, Chlorhexidine

Introduction:

The polymicrobial nature of the endodontic infections is considered as one of the main etiology for pulp necrosis and periradicular lesions. Successful endodontic treatment depends on various factors such as chemo-mechanical debridement, appropriate intracanal medicaments and three dimensional obturation in order to completely seal the root canal system against reinfection. Many studies have reported higher prevalence of E. Faecalis (24%-77%) cultured in root canals of teeth with failed endodontic treatment and flare ups.[1,2] . It has the ability to survive in root canal system due to its various virulence factors. The most-cited virulence factors are aggregation substance, surface adhesins, sex pheromones, lipoteichoic acid, extracellular superoxide production, the lytic enzymes gelatinase and hyaluronidase, and the toxin cytolysin.[3] Each of them may be associated with various stages of an endodontic infection as well as with periapical inflammation. While some products of the bacterium may be directly linked to damage of the periradicular tissues, a large part of the tissue damage is probably mediated by the host response to the bacterium and its products. To ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required.

The adverse effects following systemic application and the incompetence of systemic antibiotics in endodontic conditions has resulted in the use of local application of antibiotics in root canal treatment, that is within the canal system [4] . The first reported locally used antibiotic product was a polyantibiotic paste containing penicillin, bacitracin, streptomycin and caprylate sodium.[5] Taking into account that endodontic infections are polymicrobial, tetracyclines (tetracycline HCl, minocycline, demeclocycline, doxycycline), a group of broad-spectrum antibiotics that are effective against a wide range of microorganisms, have been proposed as intracanal topical antibiotics.

Triple antibiotic paste (TAP) is a mixture of metronidazole, ciprofloxacin, and minocycline. TAP has been used as an intracanal medicament for disinfecting the root canals during regenerative procedures. [7] The rationale of using TAP in this study is that the infected root canal is inaccessible to the local immune system and the concentration of the drug that reaches the canal space after administration of systemic antibiotics is minimal and unlikely to inhibit the bacterial growth. Therefore, the local application of antibiotics within the root canal system may be a more effective mode of delivering the drug.[8]

To increase the stability of intracanal medicament, chitosan can be used as a drug carrier. It has attracted attention in dental research due to its biocompatibility, biodegradability, bioadhesion and lack of toxicity, it has added advantage of slow and controlled release of intracanal medicament. [6,9,10] Chitosan is produced by the partial deacetylation of chitin which is the second most abundant natural polysaccharide composed of β (1→4) linked N-acetyl glucosamine units. Chitosan is a cationic

polymer derived from the exoskeleton of crustaceans (such as crabs). It is composed of copolymers of glucosamine and N-acetyl glucosamine. An important property of chitosan for study as an excipient is its ability to become hydrated and form gels in acidic aqueous environments and is thus used to prepare slow release drug delivery systems.

Chlorhexidine (CHX) in an alternative for root canal medication because of its broad-spectrum antimicrobial activity and high substantivity. It acts by adsorbing onto the cell wall of microorganisms causing intracellular component leakage. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on the microbial cell walls thereby altering the cells osmotic equilibrium. This increases the permeability of cell wall which allows the CHX molecule to penetrate into the bacteria.

The addition of vehicles to these intracanal medicaments not only improves the handling characteristics but also enhances diffusion through dentinal tubules, antimicrobial activity, and release of the medicaments. [10,11]

So, the aim of the present study is to evaluate the antimicrobial activity of triple antibiotic and modified double antibiotic paste with chlorhexidine and chitosan as vehicles against E.faecalis. Here in this study combination of amoxicillin clavulanate with metronidazole will be referred to as modified DAP.

MATERIALS AND METHOD

PREPARATION OF MEDICAMENTS

Group A- Modified double antibiotic paste [14] : It was prepared by removing the coating and crushing of antibiotic Amoxicillin 500mg + Clavulanic acid 125 mg (Moxclav 625mg, Sun Pharmaceutical Industries Ltd., India) and Metronidazole (Metrogyl 400 mg, J. B. Chemicals and Pharmaceuticals Ltd., India) tablet in a mortar and pestle. The powder thus obtained were weighed separately and mixed in a ratio of 1:1 by weight to obtain modified double antibiotic paste.

Group B- Triple antibiotic paste: It was prepared by removing the coating and crushing of antibiotic ciprofloxacin (Ciplox 500 mg, Cipla, India), metronidazole (Metrogyl 400 mg, J. B. Chemicals and Pharmaceuticals Ltd., India), and minocycline (Minoz 100mg, Cipla, India) tablets using a mortar and pestle. The ciprofloxacin, metronidazole, and minocycline powders thus obtained were weighed separately and mixed in a 1:1:1 proportion respectively, to obtain TAP mixture.

The crushed powder was passed through a fine sieve to remove heavy filler particles and obtain a fine powder.

PREPARATION OF THE MEDIUM FOR E. faecalis:

Standard strains of *E. faecalis* (ATCC29212) spores were procured from HiMedia Laboratories, Mumbai. The bacterium was grown and maintained on Brain Heart Infusion (BHI) broth (HiMedia Laboratories, Mumbai). To preserve the bacterium and its characteristics, cultures were frozen (-20ºC) in

vials with glycerol from which new stock cultures were periodically established. Initially, as per the manufacturer's instructions viability of spores was checked by reviving a pellet from the available vial of *E.faecalis* into 5ml of BHI broth at 37ºC for 18 hours followed by observing changes in turbidity to check bacterial growth. A spectrophotometer was used to check bacterial concentration of a drop of BHI containing *E.faecalis* placed in saline solution. The broth culture suspension of bacteria was adjusted at a density equivalent to the barium sulfate standard of 0.5 McFarland units (equivalent to $1.5x10^8$ CFU/ml) [12]

Aliquots of the suspension containing E. faecalis were spread on 15 different [13]140-mm diameter Petri dishes containing Mueller-Hinton Agar medium (Merck, Darmstadt, Germany). Excess inoculum was removed with a pipette and the inoculated plates were dried for 15 minutes at 37°C. Each plate was divided evenly into 4 sections. In each section of each plate, a well of dimension 5 mm in diameter and 4 mm in depth was created with a sterile stainless-steel cylinder. Each medicament prepared was placed in one well each.(Figure 1) All plates were incubated at 37°C under aerobic conditions, and zones of growth inhibition were measured at 24, 48, 72 hours using a plastic ruler and was recorded for each material. All statistical analyses were performed with the SPSS (PC version 10 software, IBM, NY, USA) statistical software package.

GROUP 1 : TRIPLE ANTIBIOTIC PASTE : MIX OF EQUAL WEIGHT (1:1:1) OF GROUND METRONIDAZOLE , CIPROFLOXACIN AND MINOCYCLINE

Sub Group 1.1: Triple antibiotic paste with chlorhexidine in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency.

Sub Group 1.2: Triple antibiotic paste was mixed with chitosan in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency.

GROUP 2 : MODIFIED DOUBLE ANTIBIOTIC PASTE : MIX OF EQUAL WEIGHT (1:1) OF GROUND METRONIDAZOLE , AMOXICILLIN WITH CLAVULANIC ACID

Sub Group 2.1: Modified double antibiotic paste with chlorhexidine in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency.

Sub Group 2.2: Modified double antibiotic paste was mixed with chitosan in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency.

RESULT :

The mean diameter of growth inhibition zones of E.faecalis found at different hours indicated that for Enterococcus faecalis

- Modified Double antibiotic paste (DAP) with chlorhexidine and DAP with chitosan were equally effective
- Triple antibiotic paste(TAP) with chlorhexidine and TAP with chitosan had equal effectiveness (figure 2 &3)

- Modified Double antibiotic paste irrespective of vehicle was more effective than triple antibiotic paste (figure 2 &3)
- **DAP +CHX = DAP + chitosan > TAP + CHX =TAP + chitosan**

DISCUSSION

The success of both primary and secondary endodontic infections depends on effective eradication of causative micro-organisms. A high proportion of *E.faecalis* is seen in persistent periapical lesions. The use of intracanal medicament with antimicrobial properties between appointments may completely eradicate these micro-organisms. The ideal or optimal vehicle for delivery of antibiotics in root canal should have the ability to facilitate better diffusion of medicament through dentinal tubules and anatomical aberrations. In pursuit of the best intracanal medicament and vehicle combination there have been several attempts to modify the most commonly used intracanal medicament that is calcium hydroxide. [15,16,17] . Different studies have tested calcium hydroxide with chlorhexidine and chitosan for their antimicrobial efficacy against E. faecalis. In studies by **Shaik et al (2014)** [16] , **Elsaka et al (2012)** [17] , $Ca(OH)_2$ + chitosan combination was more effective in inhibiting the growth of *E. faecalis w*hen compared with Ca(OH)2 + saline combination. **Rahman et al (2013)** [18] , **Elaka et al (2012)**[17] **Grover et al (2014)** [19] studied the antibacterial efficacy of Chitosan gel, Chlorhexidine gel and their combination against *C.albicans* and *E.fecalis* and found that combination of chlorhexidine with Chitosan was better than using plain Chlorhexidine or Chitosan. In study by **Rahman et al.** [18] it was found that even though chitosan has antimicrobial effect against C. albicans and E. faecalis, it proved to be less effective than chlorhexidine gluconate when it was used alone.

In this study, the antibacterial efficacy of triple antibiotic paste did not depend on vehicle, that is the antimicrobial efficacy of Chlorhexidine was equivalent to that of Chitosan unlike the results of the study by **Ballal et al. (2009)**,[20] in which the author compared the antibacterial efficiency of Chitosan, Chlorhexidine and their combination. The antimicrobial effect of Chitosan against *E. faecalis*, proved to be less effective than Chlorhexidine gluconate when it was used alone.

Several researchers like Jadhav *et al.*[22] and Alireza Adl[21] *et al*. have found the triple antibiotic paste to be very effective against E. faecalis and can be considered as a more powerful root canal medicament compared to calcium hydroxide pastes. Alireza Adl *et al.* [21], reported that TAP + saline combination has shown better antimicrobial effect against *E. faecalis* when compared with Ca(OH)2 + saline combination. Ca(OH)2 + saline combination was effective at a concentration of 200 µg/ml against *E. faecalis*. The largest inhibition zones were observed for the triple antibiotic mixture/saline, triple antibiotic mixture/2% chlorhexidine and minocycline/saline, and the smallest for Ca(OH)2/saline, Ca(OH)2/2%

chlorhexidine. The promising results of triple antibiotic paste has developed it as an alternative to calcium hydroxide and its combination with different vehicles to treat persistent polymicrobial endodontic infections.

But the triple antibiotic paste has its disadvantages too. Kim *et al.*[23] and Lenherr *et al.*[24] ,identified the discoloration caused by Minocycline used in tri-antibiotic paste.

The growing resistance development in E. faecalis against most of the antibiotics including ciprofloxacin, minocycline and metronidazole has made it a necessity to pursue for a new effective antibiotic combination.[25]

In our study the efficacy of modified double antibiotic paste which consisted of Amoxicillin 500mg + Clavulanic acid 125 mg (Moxclav 625mg, Sun Pharmaceutical Industries Ltd., India) and Metronidazole (Metrogyl 400 mg, J. B. Chemicals and Pharmaceuticals Ltd., India) was better than triple antibiotic paste for E. faecalis. But the change in vehicle did not have any effect on the antibacterial efficacy. Similar to our results, V. M. Jain et al.[26] in their study found Amoxicillin-Clavulanic acid combination to be most effective against *E.faecalis* compared to other combinations which were used to increase the success rate of endodontic treatment.

CONCLUSION :

In our study the efficacy of double antibiotic paste and triple antibiotic paste differed for E. faecalis. For enterococcus faecalis predominant infection modified double antibiotic paste was more effective.

In order to obtain the maximum clinical benefit of the anti-bacterial agents used in this mode, additional research should be carried out to investigate the best drug delivery form, drug substantivity and the feasibility of using drug combinations. This would be more practical if the biofilm model was a polymicrobial one. Also possible side effects in the form of sensitization, development of resistant strains or any alteration of root canal dentin surface characteristics should be investigated.

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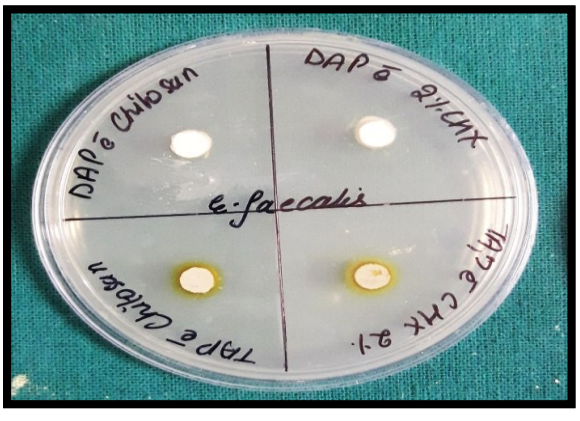
TABLES :

Table 1: The means and standard deviations of the diameters of the growth inhibition zones for E. faecalis

E.faecalis	24 HOURS Mean ±SD	48 HOURS Mean ±SD	72 HOURS Mean ±SD
TAP CHX	23.2±0.4	24.0±0.6	24.2±0.2
TAP CHITOSAN	24.4±1.2	26.2±0.6	26.3±0.4
DAP CHX	39.2±0.4	40.0±0.2	40.0±0.0
DAP CHITOSAN	40.0±0.7	40.0±0.0	40.0±0.0

Chart 1.The means and standard deviations of the diameters of the growth inhibition zones for E. faecalis



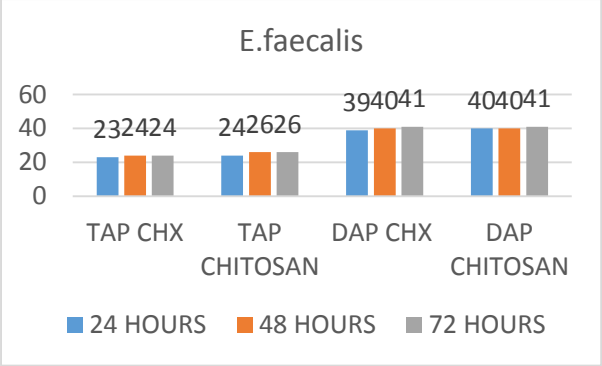


FIGURE Figure 1: MH agar plate with medicaments placed in wells

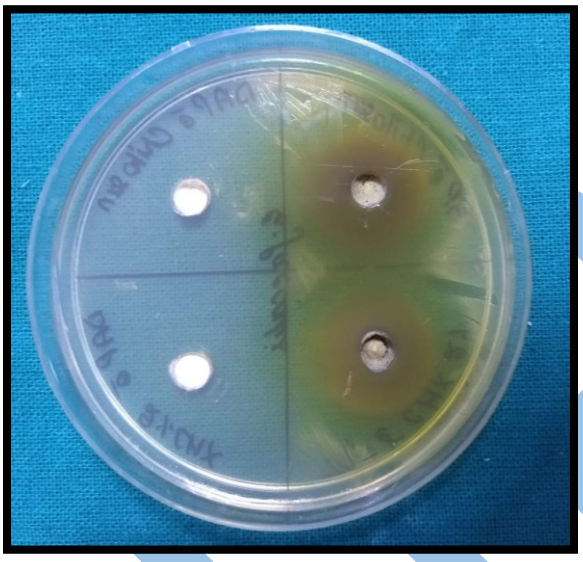


Figure 2 MH plate after E. faecalis culture

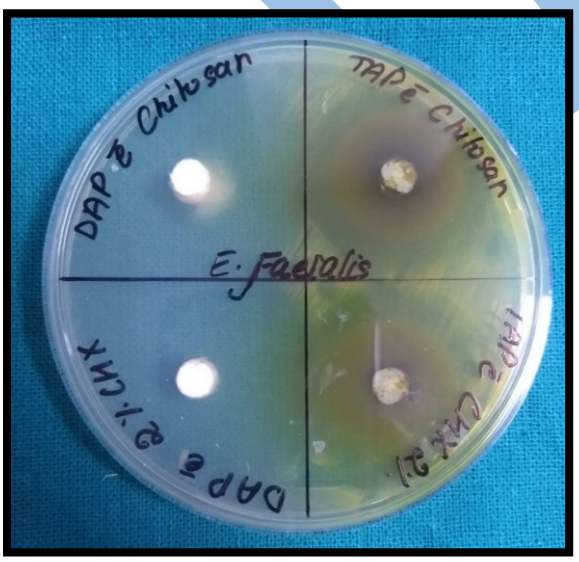


Figure 3 MH plate after E. faecalis culture showing growth on half of plate with triple antibiotic paste and no growth seen on side of modified double antibiotic paste