Original Article

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Dentistry Section

Efficacy of Triple Antibiotic Paste, Moxifloxacin, Calcium Hydroxide And 2% Chlorhexidine Gel In Elimination of *E. Faecalis*: An In vitro Study

ASHIK ALI LAKHANI[1], K.S SEKHAR[2], PANKAJ GUPTA[3], BELLAM TEJOLATHA[4], ANJALI GUPTA[5], SHRUTI KASHYAP[6], VEENA DESAI[7], SHANIN FARISTA[8]

ABSTRACT

Introduction: Root canal treatment is incomplete without usage of intra canal medicaments. They help in the reduction of bacterial count and its by-products, making canals clean and decreasing postoperative pains.

Aim: The aim of this study was to evaluate and compare the antimicrobial activity of triple antibiotic paste, Moxifloxacin, calcium hydroxide and 2% Chlorhexidine (CHX) gel in elimination of *Enterococcous faecalis (E. faecalis)*.

Materials and Methods: Seventy-five root blocks were obtained from extracted single rooted human teeth. The canal diameter was increased using Gates- Glidden drill up to size 3 and then contaminated with *E. faecalis* for 21 days. The contaminated samples were then divided into following 5 groups. Group 1: Saline (negative group), Group 2: Calcium hydroxide $Ca(OH)_2$, Group 3: 2% CHX gel, Group 4: Triple Antibiotic Paste (TAP) (50 µg – metronidazole of 400 mg, 50 µg – minocycline of 100 mg, 50 µg – ciprofloxacin of 100 mg) and Group 5: Moxifloxacin (50 µg – moxifloxacin of 400 mg). Dentin debris was obtained at the end of first, 7th, and 10th day using Gates Glidden drill sizes 4 and 5. The bacterial load was assessed by counting the number of Colony Forming Units (CFUs). The data were analyzed with the ANOVA and Post-Hoc tests to assess the differences in antibacterial efficacy between groups (p=<0.001).

Results: A 2% CHX gel alone completely inhibited the growth of *E. faecalis* after one, seven and 10 days. The 2% CHX gel was the most effective medicament against *E. faecalis*, as it showed significant differences with normal saline, calcium hydroxide, Moxifloxacin or triple antibiotic paste at all time intervals. The triple antibiotic paste group showed a moderate antibacterial effect as its difference with all group was significantly better at all days. Moxifloxacin was more effective than calcium hydroxide on 7th and 10th day.

Conclusion: Best antimicrobial efficacy was shown by 2% CHX gel. Moxifloxacin was equally efficient compared to triple antibiotic paste against *E. faecalis* at longer intervals of time.

Keywords: Antimicrobial efficacy, Dentinal tubule, Fluoroquinolone, Intra canal medicament, Root canal treatment

INTRODUCTION

Microorganisms play an important role in pulpal and periapical diseases and their elimination during endodontic treatment is essential for success of treatment [1]. The infection in the root canal system is poly microbial in nature which is dominated by anaerobes [2]. Primary root canal infection involves various bacterial species which is dominated differentially at different stages of infection [3].

The major cause of failure of root canal treatment is the persistence of microorganisms in the apical part of the root canal. Most of the microorganisms such as enterococci and streptococci are capable of surviving different conditions of filled root canals [4]. In most of the failed cases of root canal treatment teeth a Gram +ve facultative anaerobe *E. faecalis* is the frequently isolated microorganism [5]. It's because of its unique capability to invade dentinal tubules depth and surviving extreme periods of pH and limited nutrition [6].The placement of intracanal medicaments between the appointments helps in eradication of the surviving microorganism and promoting the successful outcome of the treatment [7].

Antimicrobial irrigants and medicaments are used during instrumentation in canal which reduce bacterial population effectively however some bacteria may survive in lateral and accessory root canal, isthmi and apical delta [4,8]. The most common intracanal medicament in root canal treatment is calcium hydroxide which has it's effect on most of the bacteria because of its high pH. *E. faecalis* does not responds to $Ca(OH)_2$ as it resides deeper part of dentinal tubules where pH seen to be stable because of buffering action of dentin [9].

CHX is widely used in endodontics due to its antimicrobial activity. It's used as both irrigant and intracanal medicament [10] due to its inhibitory effect on bacteria which is found in endodontic infection against Gram positive and gram negative microorganisms [11]. The efficacy of CHX is because of positively charged CHX molecule which interacts with negatively charged microbial cell wall and thereby, increasing the permeability of the cell wall and precipitation of cytoplasmic contents resulting in cell death [12].

Triple antibiotic paste is a routinely used intracanal medicament during regenerative endodontics practice. It contains mixture of ciprofloxacin, metronidazole and tetracycline derivative called minocycline. The action of this mixture against various endodontics microbes had been studied extensively and proved to be beneficial [13].

Moxifloxacin is a new fluoroquinolone with expended spectrum of activity, including anaerobes and Gram positive organisms, especially the multi-resistant ones [14]. It has been found to be one of the most active antibiotics against *E. faecalis* with lowest MIC50 and MIC90 [15].

The laboratory study was carried out to test the efficacy of triple antibiotic paste, Moxifloxacin, calcium hydroxide and chlorhexidine in elimination of *E. faecalis*.

MATERIALS AND METHODS

This study was conducted at the Department of Conservative and Endodontics in Collaboration with Department of Microbiology at

Rungta College of Dental Science and Research Bhilai, Chhattisgarh, India.

The model previously used by Haapasalo and Ørstavik was modified for this study [16].

Cleaning and Disinfection Procedure

Seventy-five (n=75) freshly extracted human teeth from department of Oral and Maxillofacial Surgery were selected. Teeth were stored in 10% formalin for 24 hours for disinfection and organic tissue fixation was used. Afterwards, these teeth were cleaned with the help of saline and middle third of each root were obtained with the help of diamond disk. Single rooted single canal teeth, closed apex, straight root were included in the study. Teeth with curved root, calcified root, double root canal and caries teeth were excluded.

Preparation of Block

The middle third of each root canal were obtained by cutting 6 mm slices from coronal and apical parts of the roots. The internal diameter of blocks was standardized with the help of Gates-Glidden drill sizes 1, 2, and 3 were used to enlarge the canal. The blocks were submerged in 17% EDTA followed by 2.5% sodium hypochlorite for 5 minutes to remove organic and inorganic debris. In order to remove the residual chemicals, the blocks were dipped in ultrasonic cleaner for 5 minutes and were autoclaved afterwards at 121^{0}C for 30 minutes then they were immersed in 1 ml of Tryptic Soy broth (TS broth) to allow better penetration of broth into dental tubules.

Contamination of Block

Culture of pure *E. faecalis* was used in this study. Each block was contaminated with micro centrifuge tube containing 1ml of TS broth and 50 µL of inoculum of *E. faecalis.* During a period of three weeks every second day each block were transferred to fresh TS broth containing *E. faecalis*. The blocks were removed irrigated with 5 ml of sterile saline at the end of incubation period. The sample were than assigned to groups (n=15) as shown in [Table/Fig-1]. The triple antibiotic paste, calcium hydroxide powder, and Moxifloxacin were mixed in paste like consistency using distilled water. The 2% CHX gel and other intracanal medicaments were carried into canal using lentulo spirals. After removal of excess material, the coronal and apical part sealed with wax than blocks were incubated with at 37^{0}C and 100% humidity.

Antimicrobial Assessment

Antimicrobial assessment was carried out at end of 1st, 7th, 10th day. Ten blocks from each group at different incubation period were taken. After ending of each incubation period the wax was removed from the root end and irrigated with 10 ml of sterile saline and subsequently dried with paper points. Gates Glidden drill size 4-5 were used to collect dentinal debris in laminar hood flow and they were transferred to micro centrifugal having 1 ml of sterile TS broth then subsequently incubated in anaerobic environment at 37°C for 24 hours. After the ending of anaerobic incubation cycle the content of each tube was serially diluted for 3 times in 100 ml of broth in 900 ml of sterile broth and at last 100 ml of this diluted sample was spread over TS broth agar and allowed to incubated for 48 hours. Colonies were counted and tabulated.

STATISTICAL ANALYSIS

ANOVA and Post-Hoc test were used to compare the difference in antimicrobial efficacy between all groups (p=<0.001).

RESULTS

The results are sequentially shown in [Table/Fig-2-7]. The saline group (negative) showed larger infiltration in dentinal tubules with *E. faecalis* colonies confirming the infection in all blocks. The 2% CHX gel was the most effective medicament against *E. faecalis*, as it showed significant differences with saline, calcium hydroxide, Moxifloxacin or triple antibiotic paste in all time intervals.

Group 1	Saline (negative group)
Group 2	Calcium hydroxide powder
Group 3	2% CHX gel
Group 4	Triple antibiotic paste 50 µg – metronidazole of 400 mg 50 µg – minocycline of 100 mg 50 µg – ciprofloxacin of 100 mg
Group 5	Moxifloxacin 50 µg – moxifloxacin of 400 mg

[Table/Fig-1]: Study groups included in the research.

	Moxi-floxocin	TAP	Calcium Hydroxide	Chlor-hexidine	Normal Saline
Mean	2.72	2.16	2.48	1.22	4.72
Standard Deviation	0.0836	0.0894	0.1303	0.1643	0.1095

[Table/Fig-2]: Comparison of mean number of *E. faecalis* colony count (10^{-2}) among various intracanal medicaments on 1st day.
F-value= 581.2, p-value= <0.001

	Moxifloxocin	TAP	Calcium Hydroxide	Chlorhexidine	Normal Saline
Mean	2.26	1.72	2.28	0.9	5.32
Standard Deviation	0.0894	0.1095	0.1303	0.1414	0.1095

[Table/Fig-3]: Comparison of mean number of *E. faecalis* colony count (10^{-2}) among various intracanal medicaments on 1st day.
F-value= 1016.9, p-value= <0.001

	Moxifloxocin	TAP	Calcium Hydroxide	Chlorhexidine	Normal Saline
Mean	2.46	2.46	2.36	1.36	8.5
Standard Deviation	0.0547	0.08944	0.0547	0.2190	0.1000

[Table/Fig-4]: Comparison of mean number of *E. faecalis* colony count (10^{-2}) among various intracanal medicaments on 10th day.
F-value= 2866, p-value= <0.001

Duration	Intracanal Medicament	Comparison with Standard Intracanal Medicaments	p-value
On 1st day	Moxifloxacin	Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	0.034
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Triple Antibiotic Paste	Moxifloxacin	<0.001
		Calcium Hydroxide	0.003
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Calcium Hydroxide	Moxifloxacin	0.034
		Triple Antibiotic Paste	0.003
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Chlorhexidine	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Normal Saline	<0.001
	Normal Saline	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Chlorhexidine	<0.001

[Table/Fig-5]: Intergroup comparison among various intracanal medicaments on 1st day using Post-Hoc test.

The triple antibiotic paste group showed a moderate antibacterial effect as its difference with all group was significant in all days. Moxifloxacin was effective against calcium hydroxide in 7th and 10th day.

Duration	Intracanal Medicament	Comparison with Standard Intracanal Medicaments	p-value
On 7th day	Moxifloxacin	Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	0.999
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Triple Antibiotic Paste	Moxifloxacin	<0.001
		Calcium Hydroxide	<0.001
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Calcium Hydroxide	Moxifloxacin	0.999
		Triple Antibiotic Paste	<0.001
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Chlorhexidine	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Normal Saline	<0.001
	Normal Saline	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Chlorhexidine	<0.001

[Table/Fig-6]: Intergroup comparison among various intracanal medicaments on 7th day using Post-Hoc test.

Duration	Intracanal Medicament	Comparison with Standard Intracanal Medicaments	p-value
On 10th day	Moxifloxacin	Triple Antibiotic Paste	1.000
		Calcium Hydroxide	0.684
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Triple Antibiotic Paste	Moxifloxacin	1.000
		Calcium Hydroxide	0.684
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Calcium Hydroxide	Moxifloxacin	0.684
		Triple Antibiotic Paste	0.684
		Chlorhexidine	<0.001
		Normal Saline	<0.001
	Chlorhexidine	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Normal Saline	<0.001
	Normal Saline	Moxifloxacin	<0.001
		Triple Antibiotic Paste	<0.001
		Calcium Hydroxide	<0.001
		Chlorhexidine	<0.001

[Table/Fig-7]: Intergroup comparison among various intracanal medicaments on 10th day using Post-Hoc test.

DISCUSSION

Various studies models are proposed for evaluation of different intracanal medicament against the resistant microflora in endodontics [8,16]. The methodology for evaluating the efficacy of intra canal medicament was adopted from the study done by Adl A et al., [17]. *E. faecalis* was selected for this study as its most commonly associated with failed root canal treated teeth [18].

The triple antibiotic paste, is a mixture of metronidazole, ciprofloxacin, and minocycline which is used as an intracanal medicament for disinfection of immature necrotic teeth, during regenerative endodontic procedures. Moxifloxacin is a synthetic fluoroquinolone of 4th generation which differ from earlier class of fluoroquinolones such as levofloxacin, ciprofloxacin having greater potential against gram positive and gram negative bacteria [19]. It has excellent bioavailability, a long half-life, and good tissue penetration [20]. CHX is commonly used as irrigant and intracanal medicament in routine endodontic procedure. It is effective against wide variety of gram positive and negative bacteria, fungus and yeasts.

Tankovic J et al., found moxifloxacin to be 2-4 times much more active than sparfloxacin, ciprofloxacin and 16 times more active than ofloxacin [21]. According to Edmiston CE et al., moxifloxacin was proved to be highly efficient against methicillin susceptible *S. aureus*, *E. faecalis* and *streptococcus* when compared with fluoroquinolones [22]. Pinheiro E et al., demonstrated high (100%) susceptibility of all the 21 isolates of *E. faecalis* to amoxicillin, clavulanic acid, moxifloxacin and vancomycin while ciprofloxacin demonstrated less susceptibility (80%) compound to the mentioned drugs [23]. Schubert S and Dalhoff A concluded the activity of moxifloxacin to be more effective than imipenem and ertapenem against *E. faecalis* in monoculture and mixed culture [24]. Al Ahmad A et al., confirmed the susceptibility of all strains of *E. faecalis* for moxifloxacin [25]. In present study, moxifloxacin was found to be less effective than triple antibiotic paste on 1st and 7th day but on 10th day the activity was comparable to triple antibiotic paste. This may be attributed because of its wider spectrum against both Gram positive and negative organisms and different mechanism of action than ciprofloxacin.

In a study, by Lindskog S et al., 2% CHX gel was active against *E. faecalis* even after 21 days of root dentin treatment [26]. Dametto et al., confirmed that 2% CHX gel and 2% CHX liquid significantly reduced the number of *E. faecalis* colonies. [27]. The most effective medicament in present study against *E. faecalis* was found to be CHX as it shows sustained activity against *E. faecalis* at different time intervals. This can be explained by contact between molecules of positive charge and phosphate group of negative charge allowing CHX molecules to enter bacterial cell wall with toxic effect. The present study showed no considerable effect of CHX on *E. faecalis* which was confirmed with previous studies about the resistance of *E. faecalis* against CHX [27]. *E. faecalis* is resistant to calcium hydroxide, especially when a high pH is not maintained [28]. This may be due to fact that, *E. faecalis* can survive a pH of 11 and that level of pH couldn't be reduced because of the buffering capacity of dentin. The resistant to pH change might also be due to the blockage of dentinal tubules by the layer of number of bacteria used in this study [29].

In the present study, triple antibiotic paste is more effective than CHX which coincides with the results of the study done by Madhubala MM et al., in which propolis and triple antibiotic paste showed higher antibacterial effects than CHX on *E. faecalis* [30]. A recent in vitro study showed that compared to CHX, the triple antibiotic paste is highly effective against *E. faecalis*. In the study done by Adl A et al., triple antibiotic paste, including metronidazole, ciprofloxacin and minocycline, had an appropriate effect on *E. faecalis* [31].

As our study was limited to the number of samples and the medicaments, more studies are needed with greater sample size for evaluating the efficacy of moxifloxacin on *E. faecalis* as an intracanal medicament in failed root canal treated cases. In coming future, we may find moxifloxacin as single drug replacement for the commonly used intracanal medicaments triple antibiotic paste and CHX, in resistant microbes of root canals.

CONCLUSION

Within the perceptive of the study it can be concluded that 2% CHX gel is the most effective medicament against *E. faecalis* in infected root canals.

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PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
2. Head, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
3. Reader, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
4. Senior Lecture, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
5. Senior Lecture, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
6. Postgraduate Student, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
7. Postgraduate Student, Department of Oral and Maxillofacial Pathology, Rungta College of Dental Science and Research Institute, Bhilai, Chhattisgarh, India.
8. Postgraduate Student, Department of Conservative Dentistry and Endodontics, Maitri Dentistry and Research Institute, Durg, Chhattisgarh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ashik Ali Lakhani,
Postgraduate Student, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Science and Research Institute, Bhilai-490001, Chhattisgarh, India.
E-mail: ashik.lakhani@gmail.com

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