

Vrunda Parikh\*, Dharati Patel\*\*, Hinal Thakkar\*\*\*, Bhumi Sarvaiya\*\*\*\*, Krunal Chokshi\*\*\*\*\*

**ABSTRACT**

**Introduction:** The success of root canal treatment is mainly based on the complete eradication of microorganisms by instrumentation and disinfection protocols followed during the procedure. Due to anatomical complexities like lateral canals, achieving long term success would be difficult because these areas have limited access to instruments and irrigants. Hence, application of intracanal medicament has been considered as an important step to reduce the persistent bacterial load inside the root canal. Since the routine intracanal solutions have the limited time for action so gels were used in the study to increase the time of action and reduce the bacterial load. **Aims and Objectives:** To evaluate antibacterial efficacy of various gel formulations when used as an intracanal medicament in primary molars. **Material and Method:** Carious primary molars with adequate coronal tooth structure were selected and randomly allotted to 4 different groups- 2% Metronidazole gel (Positive control), 2% Chlorhexidine gel, 2% Povidone-iodine gel and no medication group (Negative control). Selected tooth was anaesthetized and isolated using rubber dam. Access opening was done and canals were negotiated and biomechanical preparation was done using K-files upto #35 with frequent irrigation in between with normal saline. Pre-operative sample was taken using a paper point (#30) and was transported to lab in vial containing brain-heart infusion broth. Intracanal medicament was placed using a syringe in all the canals. Cotton pellet was placed on top of it and temporary restoration was placed. Bacterial culture of the sample was done on blood agar and colony forming units were counted. Patients were recalled after 3 days and second sample was taken immediately after removing the temporary restoration and cotton pellet using paper points and was sent for culture. Root canals were irrigated, obturated and teeth were restored with permanent restorative material. **Results:** Percentage reduction of colony forming units was calculated. Results were obtained and statistical analysis was performed using one-way ANOVA and Tukey's post-hoc test. Betadine group showed the best result among all four groups. **Conclusion:** On comparing the mean of pre-operative and post-operative CFUs, it was concluded that metronidazole, betadine and chlorhexidine showed a significant difference while no medication group showed non-significant difference.

**Keywords:** Intracanal medicament, gels, pulpectomy

**Received:** 04-05-2017; **Review Completed:** 16-08-2017; **Accepted:** 30-01-2018

**INTRODUCTION:**

Achieving predictable long-term success of root canal treatment requires effective debridement and disinfection of the root canal system.<sup>1</sup> Chemicomechanical instrumentation removes the majority of infecting bacteria, together with necrotic pulp debris.<sup>2</sup> However, this is not always achieved completely because of anatomical complexity and the limitation in accessing the canal system by instruments and irrigants. The remaining bacteria may multiply during the period between appointments, often reaching the same level that it was at the start of the previous session, in cases where the canal is not dressed with a disinfectant between visits.<sup>3</sup> Residual bacteria in obturated canals may be denied access to nutrients and die<sup>4</sup>, or they may survive and ultimately proliferate. Persistent endodontic infection may be attributed to the retention of microorganisms in dentinal tubules.<sup>5</sup> Inter-appointment intracanal medication

has been unequivocally shown to contribute to favourable outcomes when treating endodontic infections. It was observed that when no intracanal medicament was used between the appointments pathogenic microorganisms increased in number, so the need for intracanal medicaments seems to be apparent.<sup>6</sup>

Two percent chlorhexidine gluconate has been used as an irrigant and intracanal medicament in endodontics. Chlorhexidine is a bis-biguanide that acts by adsorbing onto the cell wall of microorganisms resulting in leakage of intracellular components. Chlorhexidine has a broad spectrum antimicrobial activity, targeting both gram-positive and gram-negative microbes and is biocompatible.<sup>7</sup> Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Metronidazole readily permeates bacterial cell membranes and

\*PG Student, \*\*PG Student, \*\*\* PG Student, \*\*\*\*Reader, \*\*\*\*\*Sr Lecturer

DEPARTMENT OF PEDODONTICS AND PREVENTIVE DENTISTRY, AHMEDABAD DENTAL COLLEGE AND HOSPITAL

ADDRESS FOR AUTHOR CORROSPONDENCE : DR. VRUNDA PARIKH, TEL: +91 9714964499

binds to DNA, disrupting its helical structure and leads to very rapid cell death. It has been shown to have excellent activity against anaerobes isolated from odontogenic abscesses but has no activity against aerobes.<sup>8</sup> Povidone-iodine which is used widely as a topical agent, is an iodophore in which iodine is linked to povidone, a dextran-like molecule. Povidone-iodine appears to be active against all microorganisms, including gram-positive and gram-negative, spores, mycobacteria, fungi, viruses and protozoa.<sup>9</sup>

Root canals in primary teeth relatively very thin and ribbon shaped, with large number of lateral canals. So, placing a gel based intracanal medicament will increase the duration of action of the drug by producing a sustained-release type of action. Hence, this study is aimed to assess the efficacy of chlorhexidine gel, metronidazole gel and povidone-iodine gel as intracanal medicament against *E. faecalis* in endodontically infected human primary molars.

### Materials and Methods

80 patients with necrotic or infected pulp as diagnosed clinically and radiographically who had come to the O. P. D. of Department of Pedodontics and Preventive Dentistry and who required endodontic treatment for the same. Ethical approval was obtained from ethical committee of Ahmedabad Dental College and Hospital.

### Inclusion criteria

- Patient with non-contributory medical history
- Tooth without any previous restoration
- Tooth with infected or with necrotic pulp as diagnosed clinically or radiographically
- Tooth with 2/3rd root length
- Tooth with adequate coronal structure for proper isolation, temporarization and restoration

### Exclusion criteria

- Patient with any systemic condition
- Patient with acute periapical abscess
- Retreatment cases

### Study Design

Written consent was obtained from parents of all the participants after explaining the intention of the study. Participants with carious primary molars

with adequate coronal tooth structure were selected and randomly allotted to 4 different groups.

Group I: 2% Metronidazole gel (Positive control)

Group II: 2% Chlorhexidine gel

Group III: 2% Betadine gel

Group IV: No medication placed (Negative control)

Selected tooth was anaesthetized and isolated using rubber dam. Access opening was done and canals were negotiated. Working length was obtained and confirmed by using a radiograph. Biomechanical preparation was done using K-files upto #35 with frequent irrigation in between with normal saline. Pre-operative sample was then taken using paper points (#30) and were placed in a vial containing brain-heart infusion broth and was transported to the laboratory. 0.1 ml of randomly chosen intracanal medicament was placed in each canal. Cotton pellet was placed on top of it and temporary restoration was placed on top of the cotton pellet.

### Bacterial culture

Chocolate agar plates were pre-dried in an incubator for 15-30 minutes and inoculated by streaking. A sterile wire loop of 0.01 ml was dipped into vial containing the sample and was used to inoculate on chocolate agar. Then streaking was carried out. The same procedure was done for all samples. The plates were then incubated at 37°C for 24 hours and number of colony forming units (CFU) were counted in each sample.

### Post-operative sample

Patient were recalled after 3 days and post-operative sample was taken using paper points (#30), immediately after removing temporary restoration and cotton pellet. The sample was sent to the laboratory in vials containing brain-heart infusion broth and were cultured in similar manner. The number of colony forming units in post-operative sample were counted.

After taking the post-operative sample, root canals were irrigated, dried and obturated using suitable obturating material. All the teeth were then restored with stainless steel crowns.

### Antibacterial efficacy

Percentage reduction of colony forming units was calculated and results were obtained.

Antibacterial efficacy =

$$\frac{(\text{CFU preop}^* - \text{CFU postop}^{**})}{\text{CFU preop}} \times 100$$

\*CFU preop = CFU in pre-operative sample

\*\*CFU postop = CFU in post-operative sample

Statistical analysis

The data was analysed by SPSS 20.0 software using one way analysis of variance (ANOVA), Students paired “t” test and Tukey's (P < 0.05) test. Since there are four groups, the ANOVA was used to find the significance, the multiple comparison test and pair wise comparison was done using the TUKEY post hoc test. Students paired “t” test was used for a comparison of mean between two samples of four groups.

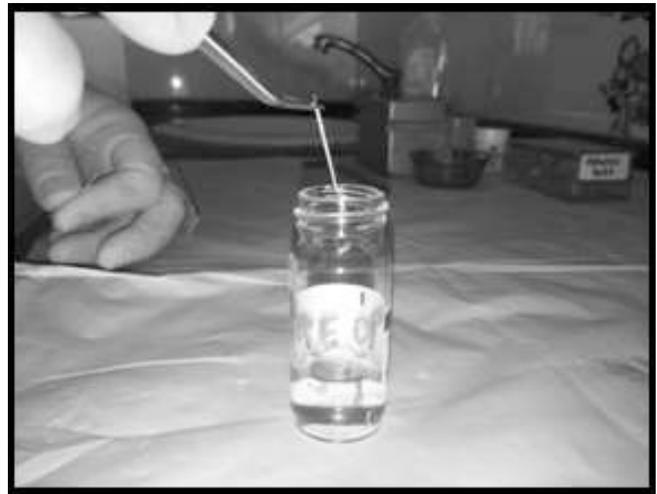


Figure 3: Placing sample in Brain Heart Infusion Broth



Figure 1: Sample collection

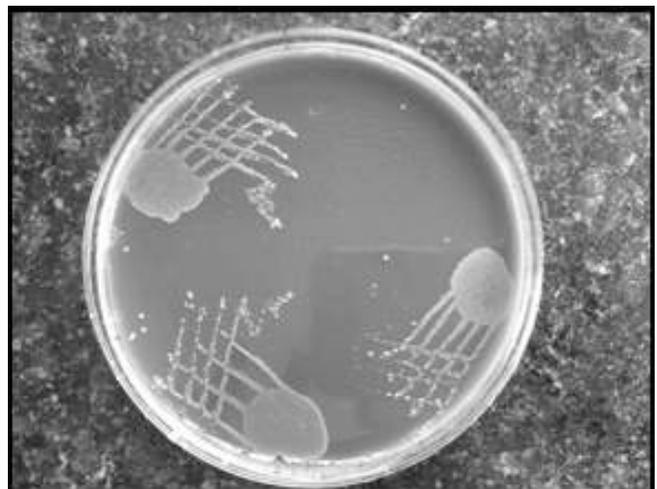


Figure 4: E. faecalis growth seen on Chocolate Agar

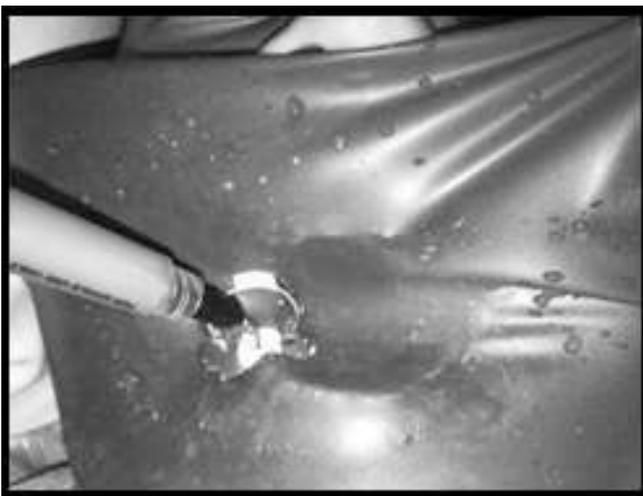


Figure 2: Medication placement

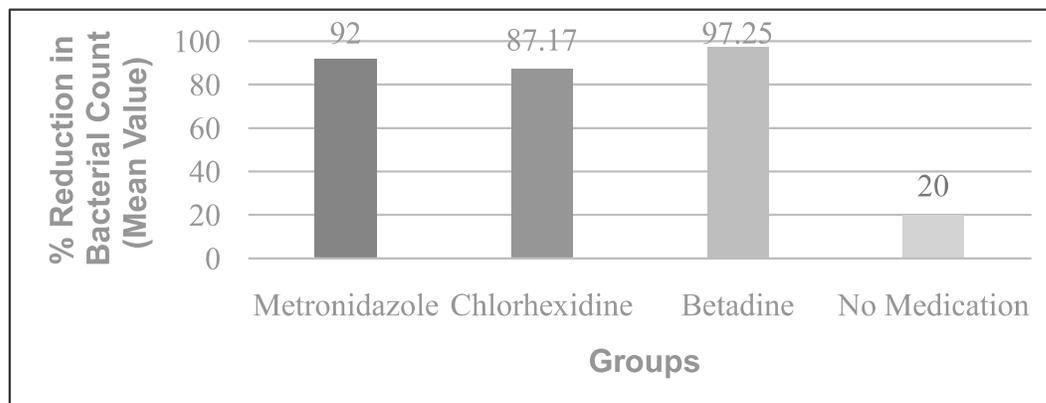
**Results:**

The results obtained after evaluating the antibacterial efficacy between all four groups – Metronidazole, Chlorhexidine, Betadine and No medication are shown table I and chart I.

<b>Table I: Mean for Percentage Reduction of Colony Forming Units of all the groups</b>				
<b>Group</b>	<b>N</b>	<b>Mean (%)</b>	<b>SD</b>	<b>p value</b>
Group I	20	92.00	25.46	<0.001(S)
Group II	20	87.17	20.24	
Group III	20	97.25	7.29	
Group IV	20	20.00	41.04	
One way ANOVA test SD: Standard Deviation P value : <0.05 (Significant), S: Significant				

<b>Table II: Intergroup Comparison of Antibacterial Efficacy</b>			
<b>Group (I)</b>	<b>Group(J)</b>	<b>Mean Difference (I-J)</b>	<b>p - value</b>
Metronidazole (I)	Chlorhexidine (II)	-4.83	0.938
Metronidazole (I)	Betadine (III)	5.25	0.923
Metronidazole (I)	No medication (IV)	-72.00	<0.001 (S)
Chlorhexidine (II)	Betadine (III)	10.09	0.625
Chlorhexidine (II)	No medication (IV)	-67.17	<0.001 (S)
Betadine (III)	No medication (IV)	-77.25	<0.001 (S)
Post hoc test SD: Standard Deviation p value : <0.05 (Significant), S: Significant			

<b>Table III: Paired Sample t test</b>							
	Mean	N	Std. Deviation	Std. Error mean	Mean Difference	% difference	P value
<b>Metronidazole (I)</b>							
Pre – operative CFU	80600.00	20	33542.43	7500.32	-72600.00	-90.07	<0.001
Post – operative CFU	8000.00	20	25464.11	5693.95			
<b>Chlorhexidine (II)</b>							
Pre – operative CFU	71500.00	20	38013.16	8500.00	-59000.00	-82.52	<0.001
Post – operative CFU	12500.00	20	20228.95	4523.33			
<b>Betadine (III)</b>							
Pre – operative CFU	75500.00	20	34864.40	7795.92	-72900.00	-96.56	<0.001
Post – operative CFU	2600.00	20	7133.32	1595.06			
<b>No Medication (IV)</b>							
Pre – operative CFU	72500.00	20	39719.41	8881.53	-11000.00	-15.17	.124
Post – operative CFU	61500.00	20	45453.85	10163.79			



**Chart I: Mean for Percentage Reduction of Colony Forming Units of all the groups**

The results showed that the percentage reduction in bacterial count was highest in betadine group and was lowest in no medication group. One way ANOVA test was applied for the comparison of mean of each group. The *p* value was found to be <0.001, which was statistically significant. On comparing the mean of pre-operative and post-operative CFUs, it was observed that metronidazole, betadine and chlorhexidine showed a significant difference while no medication showed non-significant difference. In intergroup comparison, it was seen that all the three medicament showed significant difference when compared with no medication group. However, there was no significant difference when the medication groups were compared with each other.

### Discussion

In the current study, 80 abscessed primary molars were selected which required two visit pulpectomy. Literature suggests two visit pulpectomy for abscessed cases as it provides sufficient time for the abscess to drain. The inter-appointment gap was kept to be three days as it is the usual amount of time taken by most of the practitioners and it also provides enough time for the intracanal medicament to act.

In the present study, all the pulpectomies were carried under rubber dam isolation so as to have a properly isolated field. Ahmad IA in an article in 2005 suggested that failure to use rubber dam has been shown to influence the choice of root canal irrigant, has a negative impact on treatment outcome and places the patient at risk of swallowing or aspirating materials and instruments.<sup>10</sup>

*E. faecalis* is one of the most common micro-organism which causes failure of endodontic treatment. Hancock HH and colleagues performed a study in 2001 to determine the composition of the microbial flora present in teeth after the failure of root canal therapy in North American population and concluded that out of the total sample almost 30% of the teeth had a positive culture for *E. faecalis*.<sup>11</sup> In the present study, the only irrigant that was used was normal saline as it has minimal antibacterial properties. Use of any other endodontic irrigant would have caused a bias as it would have its own bacteriocidal properties which

would alter the original results. All the three medicaments that were used were in gel form to increase the retainivity of the medication. Bhangadia MB and colleagues in a study in 2014 compared the efficacy of metronidazole gel versus metronidazole solution against *E. faecalis* in abscessed primary molars.<sup>12</sup> The results of the study concluded that metronidazole gel was more effective metronidazole solution against *E. faecalis*.

2% chlorhexidine gel was found to be 87.17% effective while 2% metronidazole was found to be 92% effective and 2% betadine was found to be 97.25% effective against *E. faecalis* in this study which is in contrast to a study done by Krithidatta J and colleagues in 2007 in which they concluded that 2% chlorhexidine gel was 100% effective while 2% metronidazole gel was 86.5% effective against *E. faecalis*.<sup>13</sup> Efficacy of betadine was compared with commonly used intracanal medicament to justify its use for a two visit pulpectomy. In a study done by Kandaswamy D and associates in 2010 antimicrobial activity of 2% chlorhexidine gel, propolis, *Morindacitrifolia* juice, 2% povidone-iodine and calcium hydroxide on *E. faecalis* was investigated and the results showed that 2% chlorhexidine was 100% effective while 2% povidone iodine was 87% effective followed by propolis (71%), *Morindacitrifolia* juice (69%) and calcium hydroxide (55%).<sup>14</sup>

Sterile paper points were used to take sample from the root canals of the selected teeth as they could reach the complete length of the canal and absorb enough fluid from the canals for culture.

Pre-sterilised sealed vials of brain heart infusion broth were used as transport medium as it allows growth of *Enterococci* and can also be used as a culture medium for the same if no other medium is available. The time lapse after the placement of sample in broth would not alter the count of *Enterococci*.

Chocolate agar was used as a culture medium for *E. faecalis*. In a study done by Gunn BA in 1984, he concluded that chocolate agar can be used as a differential medium for gram-positive cocci including streptococci, staphylococci and enterococci.<sup>5</sup>

## Conclusion

Intracanal medicaments and their use in permanent teeth has been widely demonstrated and studied, but its use and efficacy in primary teeth has not been properly evaluated. Metronidazole, chlorhexidine and betadine have been used as irrigants for decades but using them in gel form would increase its substantivity and retainivity. In this study, it was found that the bacterial count reduced upto 97.25% in betadine group followed by metronidazole group (92%) and chlorhexidine group (87.17%). The percentage reduction in *E. faecalis* count in no medication group was only 20% inspite of all patients being on systemic medication which makes it all the more necessary to use an inter-appointment

medicament in case of abscessed molars. Betadine group showed highest percentage reduction of CFUs among all the four groups. No medication group showed the least percentage reduction of CFUs of *E. faecalis*. On comparing the mean of pre-operative and post-operative CFUs, it was observed that metronidazole, betadine and chlorhexidine showed a significant difference while no medication group showed non-significant difference. In intergroup comparison, it was seen that all the three medicament showed significant difference when compared with no medication group. However, evidence for the medicaments is still insufficient for primary teeth. Further similar multicentric studies need to be carried out.

## REFERENCES:

1. Lee Y, Han SH, Hong SH, Lee JK, Ji H, Kum KY. Antimicrobial efficacy of a polymeric chlorhexidine release device using in vitro model of *Enterococcus faecalis* dentinal tubule infection. *J Endod.* 2008;34(7):855-8.
2. Manzur A, Gonzalez AM, Pozos A, Herzog DS, Friernam S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. *J Endod.* 2007;33(2):66-70
3. Bystorm A, Sundqvist G. Bacterial evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res.* 1981;89(4):321-8.
4. Peters LB, Wesenlink PR, Moorer WR. The fate and role of bacteria left in root dentinal tubules. *IntEndod J.* 1995;28(2):95-9.
5. Safavi KE, Spanberg SW, Langeland K. Root canal dentinal tubule disinfection. *J Endod.* 1990;16(5):207-10.
6. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *IntEndod J.* 1997;30(5):297-306.
7. Yesiloy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. *J Endod.* 1995;21(10):513-5.
8. Roche Y, Yoshimori RN. In vitro activity of spiramycin and metronidazole alone or in combination against clinical isolates from odontogenic abscesses. *J Antimicrob Chemother.* 1997;40(3):353-77.
9. Cherry M, Daly CG, Mitchell D. Effect of rinsing with povidone iodine on bacteraemia due to scaling: a randomized controlled trial. *J ClinPerio.* 2007;34(2):148-55.
10. Ahmad IA. Rubber dam usage for endodontic treatment: a review. *IntEndod J.* 2009;42(11):963-72
11. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in North American population. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod.* 2001;91(5):579-86.
12. Bhangadia MB, Nandlal B, Vijaykumar GS, Kulkarni PK, Shanbhog R. Clinical evaluation of sustained-release metronidazole gel versus metronidazole solution as an intracanal medicament in abscessed primary molars. *Eur Arch Paediatr Dent.* 2014;15(1):19-26.
13. Krithikadatta J, Indira R, Dorothykalyani AL. Disinfection of dentinal tubules with 2% chlorhexidine, 2% metronidazole, bioactive glass when compared with calcium hydroxide as intracanal medicaments. *J Endod.* 2007;33(12):1473-6.
14. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morindacitrifolia juice, 2% povidone iodine, and calcium hydroxide. *IntEndod J.* 2010;43(5):419-23.