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Ameya M. Joshi
Department of Conservative Dentistry and Endodontics Krishnadevaraya College of Dental Sciences, Bangalore, Karnataka, India

SM Sharath Chandra
Department of Conservative Dentistry and Endodontics Krishnadevaraya College of Dental Sciences, Bangalore, Karnataka, India

Nishtha Agrawal
Department of Conservative Dentistry and Endodontics Krishnadevaraya College of Dental Sciences, Bangalore, Karnataka, India

Correspondence
Ameya M. Joshi
Department of Conservative Dentistry and Endodontics Krishnadevaraya College of Dental Sciences, Bangalore, India

Comparison of antimicrobial activity of Mupirocin and Chlorhexidine against *Enterococcus faecalis* using agar diffusion test

Ameya M. Joshi, SM Sharath Chandra, Nishtha Agrawal

Abstract
Background: *E. faecalis* is a commonly recovered microbe in failing root canals. Due to limitations of currently popular endodontic irrigants, there is need to find novel antibacterial agents against E. Faecalis. Mupirocin is a broad spectrum antibiotic agent which is effective against gram positive organisms including MRSA. **Purpose:** The purpose of this study was to compare the antibacterial activity of Mupirocin with contemporary root canal irrigants (2% Chlorhexidine and 5% NaoCl) against *Enterococcus faecalis.* **Materials and Methods:** 6 serial dilutions of Mupirocin (1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0312%) in sterile distilled water were obtained from 2% w/w Mupirocin ointment. The anti-bacterial efficacy of these Mupirocin dilutions, 2% chlorhexidine and 5% NaOCl was tested against *E. faecalis* ATCC 29212 strain using agar diffusion method. The zone of inhibition around the wells was measured in mm using a Vernier calliper. **Results:** The zone of inhibition of 0.0312% mupirocin (6^{th} serial dilution) was comparable to 2% Chlorhexidine and hence this concentration of mupirocin was selected for comparision. The mean values for the zones of inhibition were 19.61, 21.83 and 8.67 mm respectively for 2% chlorhexidine, 0.0312% mupirocin and 5% NaOCl and the differences were statistically significant. ($p<0.05$) **Conclusion:** Based on our results, 0.0312% mupirocin may be preferable over 2% chlorhexidine as a final irrigant in the preparation of root canals.

Keywords: antimicrobial activity, Mupirocin, Chlorhexidine, Enterococcus faecalis

Introduction
Root canal morphology is complex and contains numerous ramifications and anatomical irregularities [1]. The microorganisms in root canals not only invade the anatomic irregularities of the root canal system but are also present in the dentinal tubules [2]. Persistent endodontic disease after root canal therapy may be caused by bacteria in dentinal tubules [3]. Concern has been expressed about the consequence of bacteria left in the root canal system and those that remain in the dentinal tubules [4, 5].

It is believed that the bacteria may not survive treatment, are subsequently killed, and are entombed and die from lack of nutrition, or remain viable in sufficient numbers to be a potential cause of pathosis [5]. However minimizing the number of micro-organisms in the root canal would give predictable success to endodontic treatment.

Current techniques of root canal debridement may leave areas of the root canal system completely untouched by the instruments [6]. It has also been shown that mechanical instrumentation without irrigation reduces but does not predictably eliminate bacteria in the canal [7]. Thus, a root canal irrigant is needed to aid in the debridement of the canals.

Studies have found *E. faecalis* to be a commonly recovered microbe in failing root canals [8–10]

Mupirocin is a polyketide antibiotic with broad antibacterial activity. It was isolated and characterized about 40 years ago from Pseudomonas fluorescens NCIMB 10586. Used topically it is highly effective against Gram-positive bacteria including MRSA and is used in burns patients. It binds to the isoleucyl t-RNA synthetase, resulting in inhibition of protein synthesis. As mechanism of action is different from other agents, cross resistance is unlikely [11]

This study was done to compare the antibacterial activity of Mupirocin with 2% Chlorhexidine against *Enterococcus faecalis* using agar diffusion test. As *E. Faecalis* is notorious for surviving in the root canal system and invading dentinal tubules it was chosen to test the antibacterial activity of the chelating agents in the current study.

Methods

In the pilot study 6 serial dilutions of Mupirocin (1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0312%) in sterile distilled water were obtained from 2%w/w Mupirocin ointment (Bactroban-Sigma Aldrich)

Agar diffusion test was performed according to previously described standard methodology [12] Briefly, *E. faecalis* ATCC 29212 strain was sub-cultured in 2 ml of brain heart infusion (BHI) broth and incubated at 37 degrees centigrade in a candle extinction jar for 18 hours. The culture was adjusted to 0.5 MacFarland's opacity tubes. 25 microlitres of the adjusted culture was added to 30 ml of freshly prepared BHI agar, mixed well, poured into a sterile petridish and allowed to set. Using sterile templates 3 wells of 6 mm diameter were cut in the medium and 6 such replicates were made. 100 microlitres of each 2% chlorhexidine, 5% Sodium hypochlorite (control) were added to 2 of the wells and serial dilutions of mupirocin were added to the 3rd well.

Plate was incubated at 37 degree centigrade in a candle extinction jar for 18 hours. The zone of inhibition around the wells was measured in mm using a Vernier calliper.

In the pilot study, the zone of inhibition of 0.0312% mupirocin (6th serial dilution) was comparable to 2% Chlorhexidine (Table I). Hence this concentration of mupirocin was selected to compare the antibacterial efficacy of the 2 agents.

Similar to pilot study, the 3 wells in the test plates received 100 microlitres of 0.0312% mupirocin, 2% chlorhexidine and 5% Sodium hypochlorite (control)

The test was done with 9 such replicates.

Statistical analysis was performed with Minitab version 17 statistical software (Minitab Ltd, Coventry, UK). Groups were evaluated using one-way ANOVA and Kruskal-Wallis test. Pairwise comparison was done using Newman Keuls multiple posthoc procedure and Mann-Whitney U test. All p values were two-sided and $p<0.05$ was considered statistically significant.

Results

2% chlorhexidine and 0.0312% mupirocin demonstrated large zones of inhibition; the representative zones for each of the four groups are as shown (Figure 1). 0.0312% mupirocin had zones of inhibition much larger than those of 5% NaOCl (table II). The zones of inhibition values were found to be slightly higher for 0.0312% mupirocin when compared with 2% chlorhexidine. 5% NaOCl (control) showed zones of inhibition half as large as 0.0312% mupirocin (table II). Mean, standard deviation, standard error and coefficient of variation for each group are as shown (table III). Mean values were 19.61, 21.83 and 8.67 mm respectively for 2% chlorhexidine, 0.0312% mupirocin and 5% NaOCl. Pair-wise comparative analysis showed statistically significant differences between each of the three groups (table III).

By Mann-Whitney U-test, the zones of inhibition were significantly higher for 0.0312% mupirocin when compared with 2% chlorhexidine ($p=0.0003$) and 5% NaOCl ($p=0.0003$) (table IV)

Discussion

E. Faecalis is a Gm+ve facultative anaerobic, cocci, occurs in pair or short chains. It catabolises variety of energy sources including carbs, glycerol, lactate, malate, citrate and arginine. E. faecalis is termed the root canal survivor and 'star' in post-treatment disease. It is commonly found in persistent Endodontic infection (67-77%). This Robust organism, grows in range of 10-45ºC, can withstand sodium dodecyl sulfate, bile salts, hyperosmolarity, heat, ethanol, hydrogen peroxide, acidity, and alkalinity. (13) E. faecalis possesses virulence factors that enable it adhere to dentin, compete with other bacteria, alter host response and transfer plasmids responsible for drug resistance to other bacteria. The MC4 strain of E. faecalis expresses plasmid pAMS1 which renders it tetracycline resistant making MTAD ineffective (14)

A systematic review and meta-analysis concluded NaOCl or CHX showed limited ability to eliminate E. faecalis biofilms when evaluated by either PCR or culture techniques. (15)

These limitations of currently popular endodontic irrigants prompts the need to find more efficient antibacterial agents against E. Faecalis.

Mupirocin is an antimicrobial belonging to monoxycarbolic acid class originally isolated from Pseudomonas fluorescens. Used topically it is highly effective against Gram-positive bacteria including MRSA, oral streptococci.

Mupirocin is a mixture of several pseudomonic acids, with pseudomonic acid A (PA-A) constituting greater than 90% of the mixture. It reversibly binds to the isoleucyl t-RNA synthetase resulting in inhibition of protein synthesis. DNA and cell wall formation are also negatively impacted. (16)

This study shows that the antibacterial activity of 0.0312% and higher concentrations of mupirocin against *E. faecalis* is significantly superior to that of 2% chlorhexidine and 5% NaOCl. Another potential advantage over Chlorhexidine is that Mupirocin is not affected by tissue fluids and presence of organic debris.

Mupirocin is available as 2% thixotropic gel which can be used as an inter appointment medicament or as a final irrigant in solution form in retreatment cases. Based on our results, 0.0312% mupirocin may be preferable over 2% chlorhexidine as a final irrigant in the preparation of root canals.

Tables and figures

Table I: Pilot study: Zones of inhibition (in mm)

Replicates	2% Chlorhexidine	Mupirocin		5% NaOCl
		%	mm	
1	20	1	25	8.5
2	21	0.5	24.5	9
3	20	0.25	23.5	9
4	19	0.125	23	8
5	20	0.0625	22.5	8.5
6	19	0.0312	21	8.5

Table II: Zones of inhibition (in mm)

Replicates	2% Chlorhexidine	0.0312% Mupirocin	5% NaOCl
1	19.5	21	9
2	20	22.5	8.5
3	19	22	9
4	20	22.5	8.5
5	19.5	21.5	9
6	20.5	22	8.5
7	19	21	9.5
8	19.5	22.5	8
9	19.5	21.5	8.5

Table III: Pair wise comparison of three groups with zones of inhibition (in mm) by Newman Keuls multiple post hoc procedures

Groups	2% Chlorhexidine	0.0312% Mupirocin	5% NaOCl
Mean	19.61	21.83	8.61
Standard deviation	0.49	0.61	0.33
Standard error	0.16	0.2	0.11
Coefficient of variation	2.48	2.8	3.87
2% Chlorhexidine	-		
0.0312% Mupirocin	p=0.0001*	-	
5% NaOCl	p=0.0001*	p=0.0001*	-

*p<0.05

Table IV: Pair wise comparison of four groups with zones of inhibition (in mm) by Mann-Whitney U test

Groups	Mean	SD	Median	Sum of ranks	U-value	Z-value	p-value
2% Chlorhexidine	19.61	0.49	19.50	45.00			
0.0312% Mupirocin	21.83	0.61	22.00	126.00	0.00	-3.5762	0.0003*
2% Chlorhexidine	19.61	0.49	19.50	126.00			
5% NaOCl	8.61	0.33	8.50	45.00	0.00	-3.5762	0.0003*
0.0312% Mupirocin	21.83	0.61	22.00	126.00			
5% NaOCl	8.61	0.33	8.50	45.00	0.00	-3.5762	0.0003*

*p<0.05

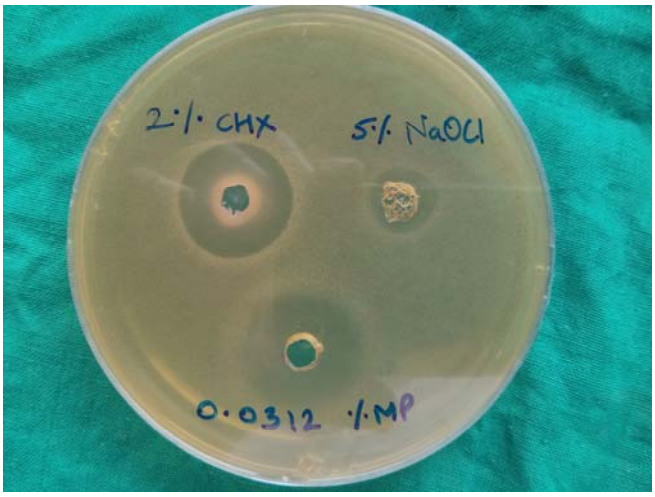

Fig 1: Representative image for zones of inhibition for the three groups. CHX, 2% chlorhexidine; 5% NaOCl, 0.0312% mupirocin

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