

Lesion Sterilization and Tissue Repair (LSTR)-3mix MP Therapy showed Reliable Efficacy against the Most Resistant Endodontic Bacteria *Enterococcus faecalis*

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Abstract :

Sterilization of bacteria in root canal system is one of the prominent problems. Some bacteria could remain in root canal even after using conventional medicaments. Evidences suggested that *Enterococcus faecalis* (*E. faecalis*) caused substantial root canal infections. So, elimination of such organism is important to achieve treatment success.

Research hypothesis: Sterilization of root canal with Lesion Sterilization and Tissue Repair (LSTR)-3mix MP Therapy is thought to be more effective against *E. faecalis* comparing to single antibiotic.

Methods: In-vitro cross-sectional with interventional type of study to observe the zone of inhibitions by *E. faecalis* culture using LSTR-3mix (metronidazole, ciprofloxacin and minocycline) and calcium hydroxide (control).

Results: The results strongly recommended that LSTR-3mix sufficiently able to inhibit *E. faecalis* growth.

Key words : Enterococcus faecalis, LSTR-3 mix MP therapy, zone of inhibitions.

Introduction :

Sterilization of microorganisms is one of the most important parts for the successful root canal treatment. The effects of bacteria on pulp may result from bacterial toxins that continuously insult the pulp and may cause irreversible damage even necrosis of pulp.^{1,2} The objectives of root canal treatment are elimination of bacteria, pulp and related irritants from root canal system and mechanical procedures such as reaming, filing, cleaning and shaping followed by 3D obturation.² It has been reported that *E. faecalis* caused substantial root canal infections.³

It may remain in the canal with or without necrotic tissues as well as dentinal tubules, accessory canals and lateral or apical delta that may cause pulpal and periapical inflammation.⁴

E. faecalis is a normal inhabitant of the oral cavity.³⁻⁵ *E. faecalis* is associated with different forms of periradicular diseases including primary endodontic infections and persistent infections.³ In the category of primary endodontic infections, Enterococcus faecalis is associated with asymptomatic chronic periradicular lesions. *E. faecalis* is found in 4 to 40% of primary endodontic infections.³

The frequency of *E. faecalis* found in persistent periradicular lesions has been shown to be much higher. In fact, failed root canal treatment (RCT) cases are nine times more likely to contain *E. faecalis* than primary endodontic infections³ and patients receiving endodontic re-treatment when compared to those with no Endodontic history⁵. *E. faecalis* are well adapted for survival and persistence in a variety of adverse environments.^{3-5,14}

To reduce the risk of RCT failure some medicaments were recommended by previous studies that could eliminate microorganisms from root canals even dentinal tubules.⁶⁻⁸ Elimination of all pathogenic bacteria including resistant organism *Enterococcus faecalis* should be essential in order to achieve successful treatment. In case of root canal sterilization /cleaning, different intra-canal medicaments are used in Dentistry.^{6-9,14} Recently, LSTR-3mix MP therapy has been proposed to fulfill the bacteria free zone in the root canal. LSTR-3mix therapy is a new biologic approach in the treatment of carious lesions with or without pulpal and periapical involvement using a mixture of three antibiotics.^{6,7} This concept was developed by the Cariology Research Unit of the Niigata University School of Dentistry in 1988. LSTR technique involves the use of three broad spectrum antibiotics namely metronidazole, ciprofloxacin and minocycline. The 3 antibiotics are mixed with Macrogol and Propylene Glycol (MP)- which is found to be an excellent vehicle to carry 3mix into the entire dentin and through the dentinal tubules and kill all the bacteria in lesions. Preparation of LSTR-3mix is bactericidal to aerobic bacteria and anaerobes.⁶ Obligate anaerobes are sensitive to metronidazole which has wide bactericidal spectrum against anaerobes.⁷ However, since not all bacteria can be eliminated by metronidazole alone⁸, other drugs may be necessary to sterilize the infected dentin. They have added ciprofloxacin and minocycline.⁷

LSTR-3mix MP therapy could kill all bacteria taken from carious lesions, necrotic pulps, infected root dentine and endodontic lesions of permanent teeth^{8,9} and deciduous teeth¹⁰. 3mix did not cause any observable pathological changes when placed onto human pulp tissue.¹¹ In Bangladesh perspectives, clinical practice needs to be easy, cheap and convenient. A large population and dental surgeon ratio might be minimized by such effective treatment procedure.

Materials and Methods:

Study Materials:1) *E. faecalis* ATCC 29212. 2) Antibiotic disks were made from tablet metronidazole, ciprofloxacin and capsule minocycline, with maintaining the proper ratio by using standard biochemical formula.⁶ LSTR-3 mix MP and control calcium hydroxide [$\text{Ca}(\text{OH})_2$] in sterile saline pastes were prepared for soaked sterile paper disk which were placed in Mueller Hinton (MH) agar Media.

Study procedure: The strains were individually inoculated into tubes containing 5 ml of sterile 0.85% saline solution. The suspensions were adjusted with the turbidity of a McFarland 0.5 tube. Five hundred (500) μl of each test microorganism suspension were inoculated into pre set Mueller Hinton (MH) agar media. The sugar coats of metronidazole and ciprofloxacin tablets were removed with a scalpel. Each tablet was pulverized using mortar and pestle. Minocycline capsule powder was segregated. The 3 antibiotics were mixed together at the 1:1:1 ratio to prepare 3-mix. The powdered antibiotics were stored and sealed in air-tight containers. Then the 3-mix was mixed with MP (macrogol and propylene glycol) as diluents until a creamy consistency has obtained. $\text{Ca}(\text{OH})_2$ paste was prepared using calcium hydroxide powder (Mallinckrodt, Inc., Phillipsburg, NJ, USA) with sterile normal saline at a ratio of 3:1. The consistency of the paste was similar to the toothpaste.

Each disk was 4 mm in diameter and thickness was Whatman number 3. The disks were sterilized in autoclave and dried in hot-air oven at 80°C for 30 minutes. Readymade metronidazole and ciprofloxacin disks were obtained commercially from Oxoid, UK. Rest of the disks were manually made with minocycline, LSTR-3mix MP and Control $\text{Ca}(\text{OH})_2$ in Microbiology and Immunology laboratory which were prepared by appropriate mixture of drugs and solutions. Paper disk of LSTR-3mix MP and Control $\text{Ca}(\text{OH})_2$ with sterile saline paste were placed onto Mueller Hinton (MH) agar Media. After 24 hours of incubation, the zone of inhibition within microbial growth of *E. faecalis* around the disks was measured and the inhibitory zone was considered to be the shortest diameter (in mm) of the outer margin of the zone of inhibition around the disk.

Results:

Presented the data of zone of inhibition of *E. faecalis*. The diameter of zone of inhibition in metronidazole (metro) group showed no inhibition. However minocycline (mino), ciprofloxacin (cipro), LSTR-3mix MP therapy and the control $\text{Ca}(\text{OH})_2$ in normal saline showed mean of zone of inhibitions were, 24.83 mm, 28.78 mm, 50.17 mm and 5.72 mm, respectively.

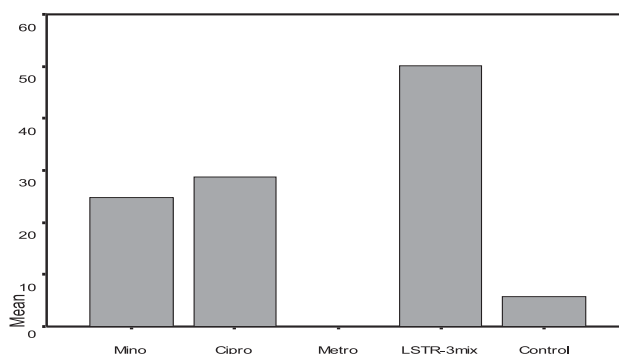
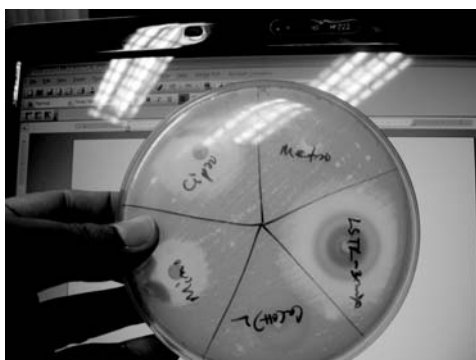


Fig -1: Mean of all drugs shows in vertical bar (In millimeter).

Since the mean of LSTR 3mix was the largest among all zones of inhibition, meant that it provided the best result. However, standard deviation (SD) measured the dispersion of data around the central value (mean) which depicts that the LSTR-3mix was quite dispersed comparing to that of other single antibiotic and control.



Pic.1: Antibiotic disks were placing on inoculated *E. faecalis* growth.



Pic.2: Largest zone of inhibition by LSTR-3mix MP therapy.

As a result, we needed to test further for proving LSTR-3mix MP therapy as the best medicaments as expressed in the hypothesis. Statistical analysis was done using paired t-test to justify it. Paired t-test is opted when two samples of one variable were dependent on each other.¹² The variable was the zone of inhibitions. The LSTR-3mix MP therapy consisted of minocycline, metronidazole and ciprofloxacin and the others were minocycline, metronidazole, ciprofloxacin and control, respectively.

After the t-test, it has been proved that P-value $0.000 \leq$ Level of Significance 0.05 . Here tabulated value is $t_{(n-1), \alpha/2}$. If $t_{cal} \geq t_{(n-1), \alpha/2}$ then H_0 will be rejected. Since calculated value was 19.359, 44.754, 29.192 and 36.961 which was greater than the tabulated value- 2.898 for $df=17$ (Mozammel H, et al. 2009), so, Null Hypothesis was rejected at .05% level of significance and Alternate Hypothesis was accepted. Again P-value $0.000 \leq$ Level of Significance 0.05 , so H_0 was rejected. [Null Hypothesis- H_0 , Level of significance- α , Calculation- t_{cal}].

Table-1: Analysis of data between LSTR 3mix therapy and other single antibiotic, based on zone of inhibition of *E. faecalis*.

	Paired Differences						t	df	Level of Significant. (2-tailed)
	Mean	Standard Deviation (SD)	Standard Error Mean (SE)	95% Confidence Interval of the Difference					
				Lower	Upper				
LSTR 3mix Vs Mino	25.33	5.55	1.31	22.57	28.09	19.359	17	.000	
LSTR 3mix Vs Metro	50.17	4.76	1.12	47.80	52.53	44.754	17	.000	
LSTR 3mix Vs Cipro	21.39	3.11	.73	19.84	22.93	29.192	17	.000	
LSTR 3mix Vs Control	44.44	5.10	1.20	46.98	41.91	36.961	17	.000	

Paired T test

Degree of freedom-df

Test statistic- t

Discussion:

Resistant bacteria *E. faecalis* may result Root Canal Treatment (RCT) failure. It may impregnate in tiny dentinal tubules. It usually not killed by most of medicament or any single antibiotic. Several studies have been performed by using LSTR and found excellent clinical results. In the permanent teeth^{8,10,13} reported that the bactericidal effect of LSTR-3mix is able to sterilize the lesion. Furthermore, these studies also noted that LSTR-3mix MP therapy could kill all bacteria taken from carious lesions, necrotic pulps, infected root dentine and endodontic lesions of permanent and deciduous teeth.¹⁰ LSTR-3mix does not cause any pathological changes when placed in to human pulp tissue.¹¹

The present study shows that LSTR-3mix MP therapy has significant antibacterial effect on *E. faecalis* when compared to single antibiotic. This study may correspond to Tamanna A, et al. (2005)¹⁴. According to Tamanna A, et al. *E. faecalis* often revealed both intrinsic and acquired types of antimicrobial resistance properties against various antibiotics.¹⁴ Two antibiotics of LSTR-3mix showed susceptibility with *E. faecalis* except metronidazole. It was also useful in pulpotomy and pulpectomy for primary or premature permanent teeth.⁸

Therefore, it can be considered that LSTR-3mix therapy could be effective in root canal sterilization and retreat the RCT failure case by eliminating the *E. faecalis*. Further clinical study is needed to confirm the results.

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