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Comparative Evaluation Of Antibacterial Efficacy Of Metapex, Metapex With Nanosilverfluoride, Metapex With Chitosan Against Streptococcus Mutans And Enterococcus Faecalis As Obturating Material:An Invitro Study

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Abstract:
Background*: Successful endodontic treatment in primary teeth relies heavily on effective elimination of pathogenic microorganisms, particularly Streptococcus mutans and Enterococcus faecalis, which are commonly implicated in persistent root canal infections. Metapex, a calcium hydroxide and iodoform-based paste, is widely used as an obturating material due to its resorbable and antimicrobial properties. However, limitations in its spectrum of antimicrobial action have led to the exploration of enhanced formulations. Nanotechnology-based additives such as Nanosilverfluoride and Chitosan have shown promising antibacterial effects due to their high surface area and sustained release capabilities. Incorporating these agents into Metapex may improve its efficacy against resistant endodontic pathogens.*
Materials and Methods: This in vitro study was conducted in the Department of Pediatric and Preventive Dentistry, Davangere, in collaboration with Maratha Mandal Dental College, Belgaum. Streptococcus mutans (MTCC 890) and Enterococcus faecalis (MTCC 3159) strains were used. Three obturating materials were tested: Metapex, Metapex with Nanosilverfluoride (NSF), and Metapex with Chitosan, prepared in a 3:1 ratio. The antibacterial efficacy was assessed using the agar diffusion method. Standardized wells were filled with test materials and incubated anaerobically. Zones of inhibition were measured after 24 hours using a Vernier calliper to evaluate antimicrobial activity.
Results: Metapex showed the largest inhibition zone against Streptococcus mutans (mean: 37.73 mm), followed by NSF (23.73 mm) and Chitosan (14.33 mm). In contrast, for Enterococcus faecalis, NSF had the highest zone (34.47 mm), followed by Chitosan (25.60 mm), and Metapex (14.80 mm). ANOVA and Tukey's post-hoc tests confirmed significant differences between all groups ($p < 0.001$). NSF significantly enhanced the efficacy of Metapex against Enterococcus faecalis, a more resistant organism, while plain Metapex was more effective against Streptococcus mutans.
Conclusion: The study concluded that while Metapex alone is highly effective against Streptococcus mutans, its efficacy against Enterococcus faecalis improves markedly when combined with Nanosilverfluoride. NSF exhibited the most balanced and broad-spectrum antibacterial action. Metapex with Chitosan showed moderate effectiveness, particularly against Enterococcus faecalis. These findings suggest that modifying obturating materials with Nanosilverfluoride or Chitosan may enhance their antimicrobial potential and clinical applicability in pediatric endodontics.
Key Word: *Antibacterial efficacy, Agar diffusion method, Chitosan, Enterococcus faecalis, Metapex, Nanosilverfluoride, Obturating material, Streptococcus mutans, Pediatric endodontics, Primary teeth*

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I. Introduction

Pulp therapy preserves primary teeth by removing infected pulp[1]. *E. faecalis* resists treatment and survives in root canals. *S. mutans*, a key caries pathogen, thrives in sucrose-rich environments and is inhibited by fluoride.

Pulpectomy is preferred for primary teeth with endodontic infections[2,3,4]. Early loss of these teeth can cause malocclusion and eruption issues, so retaining them until exfoliation is crucial [5,6].

Ideal root canal filling materials should be bacteriostatic, resorbable, non-irritating, radiopaque, and easy to insert/remove. No current material meets all criteria. Zinc oxide eugenol and calcium hydroxide are common but have limitations. Calcium hydroxide-iodoform paste (e.g., Vitapex) is safe and resorbable but may cause internal resorption[7,8,9].

Chitosan is biocompatible and antimicrobial, disrupting bacterial membranes and supporting biomedical use[10]. Chitosan nanoparticles (CS NPs), by virtue of their charge and size, are expected to possess enhanced antibacterial activity. In addition, Chitosan possesses several characteristics such as being nontoxic toward mammalian cells, colour compatibility to tooth structure, cost effectiveness, availability, and ease of chemical modification[11].

Silver nanoparticles (AgNPs), or nano-silver, are nanoparticles of silver of between 1 nm and 100 nm in size containing 20 to 15,000 silver atoms. They have been one of the most popular topics of study in recent decades because of their outstanding antimicrobial activity even at low concentrations including antibacterial, antifungal, antiviral, and anti-inflammatory effects[12]. Silver nanoparticles (AgNPs) penetrate cell walls, disrupt DNA, and are highly effective against *S. mutans* due to their small size[13].

II. Material And Methods

The study was done in the Department of Pediatric and Preventive Dentistry, College of Dental Sciences, Davangere in collaboration with Maratha Mandala Dental College, Belgaum. Microbial type culture collection (MTCC) Chandigarh from where the maintained strains of Streptococcus mutans (MTCC 890) and Enterococcus faecalis (MTCC 3159) were collected. Ethical clearance was obtained from institutional review board of the college for the study.

Study Design: An in vitro comparative study

Study Location: Department of pediatric and preventive dentistry College of dental sciences Davangere.

Study Duration: 6months

Sample size: 90 samples.

Sample size calculation: The sample size was estimated to be 15 per each group, based on a 95% confidence level, an observed standard deviation of 0.2, and a permissible error margin of 10%.

Subjects & selection method:

Metapex(MetaBiomed), Chitosan (Vedayukt India pvt limited), Nanosilverfluoride (Vedayukt India pvt limited), Agar plate with Muller Hinton agar,

Group A (Streptococcus mutans)
A1-Metapex (control group)
A2 -Metapex with nanosilverfluoride (experimental group)
A3-Metapex with chitosan (experimental group)

Group B (Enterococcus faecalis)
B1-Metapex (control group)
B2-Metapex with nanosilverfluoride (experimental group)
B3-Metapex with chitosan (experimental group)

Procedure methodology

About 150 mg of Metapex was diluted in 1 mL of DMSO (dimethyl sulphoxide). To which, 50 mg of silver fluoride nanoparticle (M+SF) and Chitosan nanoparticle (M+C) were added separately in each microcentrifuge tube. After vigorous mixing of both the samples using a vortex, the sample was taken as a test compound. Metapex mixed with DMSO was considered as control (M).

Preparation of S. mutans and E.Faecalis cultures:

Preparation of broth and bacterial growth:

The antimicrobial activity of obturating materials used in primary teeth against streptococcus mutans and Enterococcus faecalis was evaluated in this study by agar diffusion method. The standard bacterial strain of

streptococcus mutans (MTCC890) and enterococcus faecalis (MTCC3159) obtained from microbial type culture collection (MTCC) Chandigarh. The purity of test strain was confirmed using the Gram's stain. Only 0.37 grams of Brain Heart Infusion (BHI) broth was to 10mL of distilled water and mixed by gently shaking the container. This mixture was sterilized by autoclaving for 15 minutes at 121°C and 15lb pressure and then allowed to cool at room temperature. The bacterial strains are inoculated in BHI broth and incubated at 37°C for 24 hours. Following incubation, the cultures were centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was discarded and the precipitate containing microbial cells was separated from the base of test tube. The precipitate containing microbial cells was re-suspended in saline and turbidity of this culture suspension was adjusted until it is equivalent to the no.1 McFarland Standard (Approximately 3 X 108 cells/mL).

Preparation of culture medium:

Mueller Hinton agar (15.2gm) was added to 400mL of distilled water and mixed by gently shaking the container. This mixture was sterilized by autoclaving for 15 minutes at 121°C and 15lb pressure. After 15 minutes, the liquid was cooled to room temperature. In a laminar flow chamber, this liquid medium was poured in 20 petri dishes of size 90mm and allowed to set1 Inoculation of bacterial strain on culture media: Each agar plate with 20mL of Mueller Hinton agar was inoculated with 0.1mL microbial suspension using sterile swab. The 3 wells (4mm of depth X 6mm of diameter) were made in each of the agar plates, i.e., total 60 wells were prepared in 20 agar plates to test two different obturating materials and a control.

Placement of obturating materials and incubation:

Each freshly mixed experimental obturating material was placed in 10 wells of different petri dishes. These agar plates were then incubated at 37°C under anaerobic conditions in an incubator using Microaerophilia (candle jar system). An anaerobic indicator tablet (Anaeroindicator tablets) was placed in the jar to monitor oxygen contamination of the environment.

Measuring the size of zone of inhibition:

A lack in bacterial colonization was observed for each obturating material. It was indicated by growth inhibitory zones (clearing of agar) around each obturating material. The most uniform diameter segment of the zone of inhibition was determined in millimeters by measuring the shortest distance between the outer margin of the well and initial microbial growth after 24 hours of incubation.

Statistical analysis

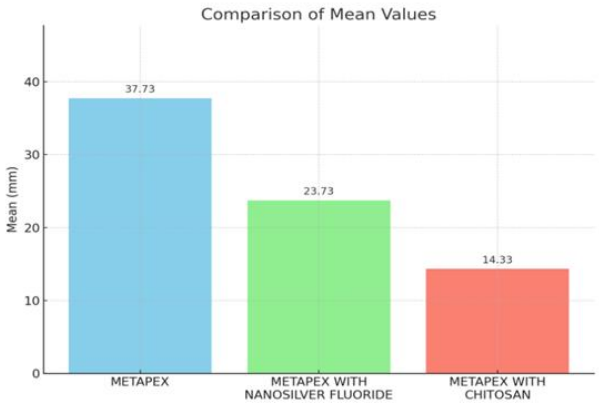
The data obtained was statistically analyzed by ANOVA, POST -HOC analysis

III. Result

TABLE 1: Explains mean value, standard deviation, standard error, 95% Confidence Interval for Mean Streptococcus mutans.

Streptococcus mutans								
	N	Mean (mm)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Metapex	15	37.73	7.658	1.977	33.49	41.97	28	55
Metapex With Nanosilver Flouride	15	23.73	2.492	.643	22.35	25.11	20	28
Metapex With Chitosan	15	14.33	2.526	.652	12.93	15.73	10	18

GRAPH 1: mean zone of inhibition for streptococcus mutans between groups



The Metapex group exhibited the highest mean zone of inhibition, with a mean value of 37.73 mm and a standard deviation of 7.658 mm. The 95% Confidence Interval (CI) for this group ranged from 33.49 mm to 41.97 mm, with individual inhibition zones ranging between 28 mm and 55 mm.

The Metapex with Nanosilver Fluoride group showed a moderate inhibitory effect, with a mean zone of 23.73 mm and a standard deviation of 2.492 mm. The 95% CI for this group was 22.35 mm to 25.11 mm, with the minimum and maximum inhibition zones recorded as 20 mm and 28 mm, respectively.

In contrast, the Metapex with Chitosan group displayed the least antimicrobial activity, with a mean inhibition zone of only 14.33 mm and a standard deviation of 2.526 mm. The 95% CI ranged from 12.93 mm to 15.73 mm, with a minimum of 10 mm and a maximum of 18 mm.

TABLE 2: Explains ANOVA of Streptococcus mutans zone of inhibition among three groups.

Streptococcus mutans					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	4159.600	2	2079.800	87.597	<0.001*
Within Groups	997.200	42	23.743		
Total	5156.800	44			

*<0.05 considered statistically significant

One-way Analysis of Variance (ANOVA) revealed a statistically significant difference in the *Streptococcus mutans* inhibition zones across the three groups. The between-group sum of squares was 4159.600 with 2 degrees of freedom, resulting in a mean square value of 2079.800. The F-value was 87.597 with a p-value of <0.001, indicating that the differences among group means were highly significant ($p < 0.05$).

Table 3: Explains Multiple Comparisons Of Streptococcus Mutans Zone Of Inhibition Using Post-Hoc Tukey.

Dependent Variable: Streptococcus Mutans						
Tukey Hsd						
					95% Confidence Interval	
(I) Groups	**(J) Groups**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**Lower Bound**	**Upper Bound**
Metapex	**Metapex With Nanosilver Flouride**	14.000*	1.779	<0.001*	9.68	18.32
	Metapex With Chitosan	23.400*	1.779	<0.001*	19.08	27.72
Metapex With Nanosilver Flouride	**Metapex**	-14.000*	1.779	<0.001*	-18.32	-9.68
	Metapex With Chitosan	9.400*	1.779	<0.001*	5.08	13.72
Metapex With Chitosan	**Metapex**	-23.400*	1.779	<0.001*	-27.72	-19.08
	Metapex With Nanosilver Flouride	-9.400*	1.779	<0.001*	-13.72	-5.08
*. The Mean Difference Is Significant At The 0.05 Level.						

Post-hoc analysis using the Tukey HSD test further confirmed the statistical significance of the differences among all pairs of groups:

- Metapex (Group A1) v/s Metapex with Nanosilver Fluoride (Group A2): The mean difference was 14.000 mm, which was statistically significant ($p < 0.001$), with a 95% CI ranging from 9.68 mm to 18.32 mm.
- Metapex (Group A1) v/s. Metapex with Chitosan (Group A3): The mean difference was 23.400 mm, also statistically significant ($p < 0.001$), with a 95% CI from 19.08 mm to 27.72 mm.
- Metapex with Nanosilver Fluoride (Group A2) v/s. Metapex with Chitosan (Group A3): The mean difference was 9.400 mm, again statistically significant ($p < 0.001$), with a 95% CI ranging from 5.08 mm to 13.72 mm.

All intergroup differences were statistically significant with p-values less than 0.001, suggesting that the choice of additive plays a critical role in determining the antibacterial effectiveness of Metapex.

Table 4, Explains Mean Value, Standard Deviation, Standard Error, 95% Confidence Interval For Mean Of Enterococcus Faecalis

Enterococcus Fecalis								
					95% Confidence Interval For Mean			
	N	**Mean (Mm)**	**Std. Deviation**	**Std. Error**	Lower Bound	Upper Bound	**Minimum**	**Maximum**
Metapex	15	14.80	3.840	.991	12.67	16.93	5	20
Metapex With Nanosilver Flouride	15	34.47	2.326	.601	33.18	35.75	31	38
Metapex With Chitosan	15	25.60	2.874	.742	24.01	27.19	21	32

The Metapex with Nanosilver Fluoride group showed the highest mean zone of inhibition**,** with a mean of 34.47 mm and a standard deviation of 2.326 mm**.** The 95% Confidence Interval (CI) for the mean ranged from 33.18 mm to 35.75 mm**,** and the values ranged from 31 mm to 38 mm.

All intergroup comparisons were statistically significant (**p < 0.001**), highlighting that the incorporation of **Nanosilver Fluoride** or **Chitosan** into Metapex significantly enhances its antimicrobial efficacy, with **Nanosilver Fluoride** providing the greatest enhancement.

- The Metapex with Chitosan group exhibited a moderate antibacterial effect, with a mean zone of inhibition of 25.60 mm**,** a standard deviation of 2.874 mm**,** and a 95% CI ranging from 24.01 mm to 27.19 mm**.** The minimum and maximum inhibition zones observed were 21 mm and 32 mm**,** respectively**.**
- In contrast, the Metapex group demonstrated the least inhibitory effect, with a mean inhibition zone of 14.80 mm and a standard deviation of 3.840 mm**.** The 95% CI ranged from 12.67 mm to 16.93 mm**,** with individual values ranging between 5 mm and 20 mm**.**

Graph 2: Mean zone of inhibition for Enterococcus faecalis between groups

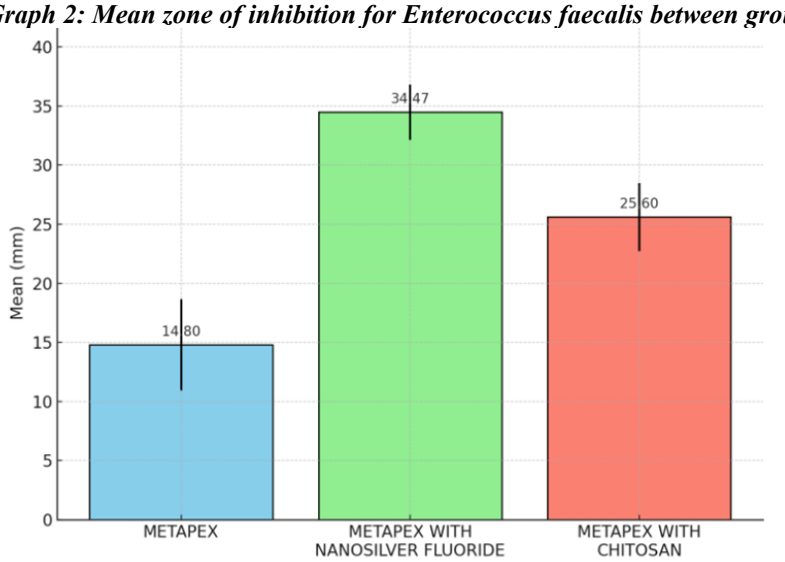


TABLE 5: Explains ANOVA of Enterococcus faecalis zone of inhibition among three groups.

Enterococcus faecalis					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2910.178	2	1455.089	153.655	<0.001*
Within Groups	397.733	42	9.470		
Total	3307.911	44			

A one-way ANOVA revealed a highly statistically significant difference in the *Enterococcus faecalis* inhibition zones across the three groups. The between-group sum of squares was 2910.178 (df = 2), with a mean square value of 1455.089. The F-statistic was 153.655 and the associated p-value was <0.001, confirming that the observed differences among group means are statistically significant **(**p < 0.05**).**

TABLE 6: Explains multiple comparisons of Enterococcus faecalis zone of inhibition among three groups.

Dependent Variable: Enterococcus Faecalis						
Tukey Hsd						
(I) Groups	**(J) Groups**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	Upper Bound
Metapex	**Metapex With Nanosilver Flouride**	-19.667*	1.124	<0.001	-22.40	-16.94
	Metapex With Chitosan	-10.800*	1.124	<0.001	-13.53	-8.07
Metapex With Nanosilver Flouride	**Metapex**	19.667*	1.124	<0.001	16.94	22.40
	Metapex With Chitosan	8.867*	1.124	<0.001	6.14	11.60
Metapex With Chitosan	**Metapex**	10.800*	1.124	<0.001	8.07	13.53
	Metapex With Nanosilver Flouride	-8.867*	1.124	<0.001	-11.60	-6.14
*. The Mean Difference Is Significant At The 0.05 Level.						

Post-hoc analysis using the Tukey HSD test showed the following significant pairwise differences:
•Metapex (GROUP B1) v/s Metapex with Nanosilver Fluoride (GROUP B2): The mean difference was -19.667 mm, which was statistically significant ($p < 0.001$), with a 95% CI from -22.40 mm to -16.94 mm.
•Metapex (GROUP B1) v/s. Metapex with Chitosan (GROUP B3): The mean difference was -10.800 mm, also statistically significant ($p < 0.001$), with a 95% CI from -13.53 mm to -8.07 mm.
•Metapex with Nanosilver Fluoride (GROUP B2) v/s Metapex with Chitosan (GROUP B3): The mean difference was 8.867 mm, again statistically significant ($p < 0.001$), with a 95% CI ranging from 6.14 mm to 11.60 mm.

IV. Discussion

Effective disinfection of primary root canals remains a major challenge due to complex anatomy and the persistence of microorganisms such as *Streptococcus mutans* and *Enterococcus faecalis*. *S. mutans* initiates caries, while *E. faecalis* is linked to persistent endodontic infections and periradicular pathologies owing to virulence factors and resistance to high pH and antimicrobial agents[14,15].

This study compared the antibacterial efficacy of Metapex, Metapex + Nanosilverfluoride (NSF), and Metapex + Chitosan.

Metapex: A calcium hydroxide–iodoform paste, Metapex exerts antimicrobial activity through high pH but shows limited effect against *E. faecalis*[16.17,18.19]. Prior studies report moderate efficacy, with better action against *S. mutans* than *E. faecalis*[20,21]. Our findings corroborate these results, showing greater inhibition of *S. mutans* (mean 37.73 mm) compared to *E. faecalis* (14.80 mm).

Metapex + NSF: Silver nanoparticles provide potent antimicrobial action by disrupting bacterial membranes and biofilms[22,23,24]. NSF demonstrated significant efficacy against resistant pathogens like *E. faecalis*, consistent with previous studies. In our study, Metapex + NSF showed the highest antibacterial activity, likely due to nanoparticle penetration and sustained ion release.

Metapex + Chitosan: Chitosan disrupts microbial membranes and exhibits bioadhesive properties enhancing retention in canals.Studies confirm its effectiveness against *E. faecalis* and biofilms[25,26,27]. In combination with Metapex, chitosan enhanced antibacterial efficacy compared to Metapex alone, though slightly less than the NSF group.

Comparative Analysis: Overall, Metapex + NSF > Metapex + Chitosan > Metapex alone. The superior effect against *E. faecalis* is particularly relevant for preventing treatment failures. Incorporating novel agents like NSF and chitosan improves the antibacterial potential of traditional obturating materials.

Strengths and Limitations: Strengths include standardized methodology, reliable microbial strains, and evaluation of clinically significant pathogens. However, in vitro conditions cannot replicate oral environment factors such as saliva, host response, or polymicrobial infections. Agar diffusion, while simple, may not reflect sustained intracanal activity.

Future Directions: In vivo studies considering biofilms, host factors, and long-term outcomes are warranted. Further exploration of nanomaterial- or biopolymer-modified obturating agents may enhance pediatric endodontic success.

V. Conclusion

This in vitro study demonstrated that Metapex alone was most effective against *Streptococcus mutans*, while its efficacy against *Enterococcus faecalis* improved significantly when combined with Nanosilverfluoride. Among the tested groups, Metapex + Nanosilverfluoride showed the highest and broadest antibacterial activity, followed by Metapex + Chitosan, with Metapex alone being least effective against resistant pathogens. Incorporating novel agents such as Nanosilverfluoride or Chitosan into conventional obturating materials may enhance disinfection in pediatric endodontics. Further clinical studies are required to validate these findings and assess long-term biocompatibility

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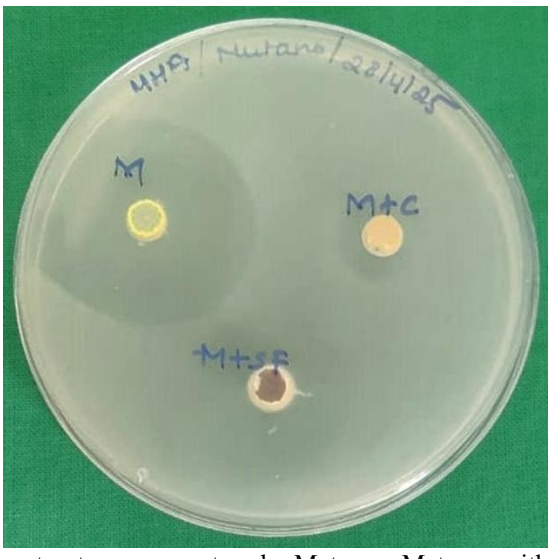

Figure 1: zone of inhibition on streptococcus mutans by Metapex, Metapex with nanosilverfluoride, Metapex with chitosan.

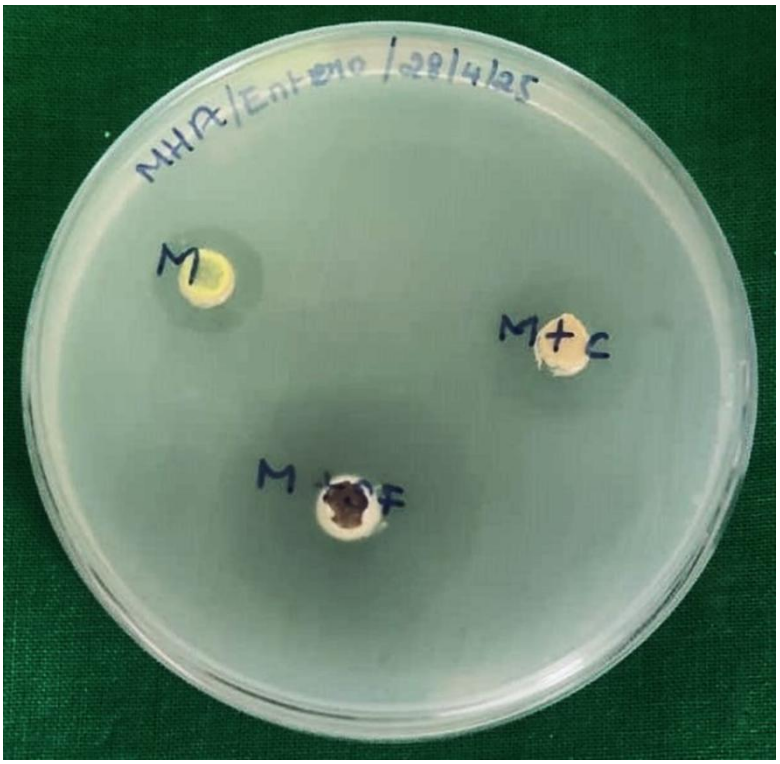

Figure 2: Zone of inhibition on Enterococcus Faecalis by Metapex, Metapex with nanosilverfluoride, Metapex with chitosan