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Comparative assessment of antimicrobial efficacy of Triple Antibiotic and Chlorhexidine Gluconate as a root-canal irrigants against E. Faecalis and C. Albicans – An in-vitro study.

Dental Science

KEYWORDS: Triple Antibiotic Solution, Chlorhexidine Gluconate, *E. Faecalis, C. Albicans.*

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ABSTRACT

Background: Key goal of successful endodontic treatment is elimination or suppression of the bacteria in the root canal.

Objective: To assess and compare antimicrobial efficacy of Triple Antibiotic and Chlorhexidine Gluconate as a root-canal irrigant against *E. Faecalis* and *C. Albicans*.

Methodology: The present in-vitro study was conducted at private medical institute, Dhule. To assess the antibacterial efficacy of triple antibiotic solution and chlorhexidine gluconate; present study was divided into 3 parts: 1) Assessment of zone of inhibition (ZOI) by well-diffusion method 2) Time-kill curve approach and 3) Mininal Inhibitory Concentration (MIC). The test organisms chosen for the present study were *E. Faecalis* and *C. Albicans* as they are the most frequently isolated species from the root canals of endodontically failed teeth. Freeze dried strains of *E. Faecalis* (MTCC 35550) and *C. Albicans* (MTCC 209) were obtained from IMTECH, Chandigarh.

Results: There was statistically no significant difference in zone of inhibition for three concentrations of triple antibiotic solution as well as chlorhexidine gluconate on *E. Faecalis* where as it was noted with *C. Albicans* at 5% of level of significance. Pairwise comparisons of ZOI showed statistically significant difference on test organisms. Kruskal Wallis test was used to find the significance of study parameters between three or more groups. Results of MIC showed that triple antibiotic solution and chlorhexidine gluconate, both were effective against test organisms. In Time-kill curve approach CHX showed no growth on both organisms whereas TAS showed growth on *C. Albicans* only after first 3 minutes.

Conclusion: From the present study it was seen that TAS was most effective against *E. Faecalis* and CHX was effective against *C. Albicans.*

INTRODUCTION

Maintaining an aseptic root canal is paramount for successful outcome of an endodontic treatment. The root canal success mainly depends on mechanical preparation, irrigation, microbial control, and complete filling of the root canal system. Microorganisms, bacteria, and their products are considered as the etiological agents in development and perpetuation of pulp and periradicular lesions. They may survive during endodontic procedures due to anatomical structural complexities and limitations of access by instrumentation and irrigants.[1]

Numerous studies have showed that endodontic re-treatment demand has increased because of increased percentage of root filled teeth with evidence of periapical infection. Pulp tissue or tissue remnants left in the root canals can serve as a nutrient source for any remaining microorganisms. This demands effective antimicrobial agents like some form of irrigation and medicaments to ensure complete elimination of intracanal microorganisms, residual tissue and to kill them for a predetermined time period.[2]

During endodontic treatment, antibiotics are a valuable adjunctiveadvised systemically or applied locally (as anirrigants and medicaments) for the management of bacterial infection. Due to potential risk of adverse effects and the ineffectiveness of systemic antibiotics in the necrotic pulpless teeth andperiradicular tissue, the local application of antibiotics may be a more effective mode for delivery in endodontics.[3]

Two most commonly isolated organisms from failed endodontic treatment and infections flare-ups *are E. Faecalis* and *C. Albicans.*[1]

Enterococcus Faecalis has the ability to survive in root canal system as a single organism without the support of other bacteria and is small enough to proficiently invade and live within the dentinal tubules.[4]

C. Albicansis one of the most common species of fungi uses dentin as a nutrient source and promotes colonization in the root canal because of its collagenolytic activity.[5]

Chlorhexidine (CHX) Gluconate is most commonly used intracanal medication for its well-known broad-spectrum antimicrobial effects. CHX molecule consists of two symmetric 4-cholorophenyl rings and two biguanide groups connected by a central hexamethylene chain. Due to the positively charged molecule, CHX interacts with negatively charged phosphate groups on the microbial cell wall and

causes its leakage.[6]

Recently a mixture of Metronidazole, Ciprofloxacin and Minocycline, also known as the triple antibiotic paste (TAP), has been used as an intracanal medicament for disinfecting the root canal during tissue regeneration.[7] Metronidazole is a wide spectrum bactericidal antibiotic. *In vitro* experiments have shown that 10 g/ml Metronidazole can eliminate more than 99% of bacteria found in infected root canals and for complete sterilization of infected root canal, we need other antibiotics such as Ciprofloxacin and Minocycline.[8] Some researchers have reported that the TAP can sterilize root dentin. Nowadays, due to increasing demand for single sitting root canal treatment in endodontic practice demands immediate disinfection of the root canal space.

Keeping this in mind an attempt was made to prepare triple antibiotic solution as an intracanal irrigant. Thus, the present in-vitro study was designed to compare the antibacterial efficacy of triple antibiotic solution with that Chlrohexidine Gluconate intracanal irrigant against *E. Faecalis and C. Albicans*

METHODOLOGY

The present in vitro study was conducted at Private Medical Institute, Dhule.

The purpose of the study was to evaluate antimicrobial efficacy of triple-antibiotic solution and chlorhexidine, solution as a root canal irrigants.

Ethical clearance for the present study in-vitro study was obtained from the institutional ethical review committee.

The following concentrations were prepared for the present study.
First, triple-antibiotic (Ciprofloxacin, Metronidazole and Minocycline) solution was prepared using API (Actual Pharmaceutical Ingredient) using ratio of 40% methanol and 60% distil water to adjust same volume (10ml) for the each concentration
1. 50grams each of Ciprofloxacin, Metronidazole and Minocycline.
2. 100grams each of Ciprofloxacin, Metronidazole and Minocycline.
3. 150grams each of Ciprofloxacin, Metronidazole and Minocycline.

Second, using commercially available 2% Chlorhexidine Gluconateirrigating solution was reconstituted using distil water to obtain following concentrations of same volume (10ml)
1. 1% Chlorhexidine solution.
2. 1.5% Chlorhexidine solution.
3. 2% Chlorhexidine solution.

The test organisms chosen for the present study were *C. Albicans*and *E. Faecalis*as they are the most frequently isolated species from the root canals of endontically failed teeth.

For the present in-vitro study thestandard strains of *E. Faecalis*(MTCC 35550)and *C. Albicans*(MTCC 209) were obtained from IMTECH Chandigarh.

To assess the in vitro antibacterial efficacy, the present study was divided in 3 parts:
1. Assessment of zone of inhibition by well diffusion method.
2. Time- kill curve approach
3. Minimal Inhibitory Concentration (MIC)

1. Well diffusion method.[9]

Strains of*E. Faecalis* and *C. Albicans*organisms were grown and suspended in 5ml of Brain heart infusion and Sabaroundi dextrose (SD) broth respectively before use for the present study.

A total of 12 agar plates (6 each for *E. Faecalis and C. Albicans*) were brought to room temperature. Before transferring colonies from the broth to plates, visually turbidity was adjusted equal to that of a 0.5 McFarland turbidity standard.

Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, asterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculumto ensure even distribution.

Inoculated plates were allowed to dry for at least 3 minutes with lid closed. With the aid of a sterile 5mm metal borer 3 equally spaced wells were bored in each agar plates aseptically.

Pre standardized volume of 0.1ml solution of triple-antibiotic and Chlorhexidine Gluconate solution of 3 different concentrations was poured in the respective wells with the help of sterile disposable dropper. Then, plates were incubated for 24hours in an incubator at 37^0C.

Using this method the experiment was done in triplicate for test organisms; then mean was taken for further statistical analysis.

Reading of plates was done only if the lawn of growth was confluent or nearly confluent, measuring diameter of inhibition zone to nearest whole millimeter from edge of the well to the periphery usingVernier caliper.

The two main trends to understand the anti-infective efficacy of antibiotics are pharmacokinetic-pharmacodynamic models; those based on the Minimum inhibitory concentration (MIC) and those based on a time-kill curve approach.

2.Time-kill curve approach-

The main concept of this approach is to understand the concentration effect relationship and dosage adjustment of a drug in a logical way and to minimize trial-and-error approaches. This approach can potentially result in substantial savings of time and expenses and may help to avoid unnecessary and, hence, unethical clinical studies.

The second method is to determine the lowest concentration of drug that prevents visible growth of the organisms is the MIC.

3. Minimal inhibitory concentration procedure.[10]

9 dilutions of each irrigant were done with BHI and SD broth separately for evaluating MIC.

In the initial tubes 20 microliter of irrigant was added into the 380microliter of respective BHI and SD broth.

For dilutions 200microliter of BHI and SD broth were added into the next 9 tubes separately.

Then from the initial tubes 200microliter was transferred to the first tubecontaining 200microliter of BHI and SD broth respectively. This was considered as 10^{-1} dilution for that particular irrigant.

From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution.

This serial dilution procedure was repeated up to 10^{-9} dilution for each irrigant.

After obtaining serial dilutions upto 10^{-9} for each irrigant, 5ul suspension of E. Faecalis and C. Albicans were taken from the maintained stock cultures and added into 2ml of BHI (brain heart infusion) and SD broth respectively.

200microliter of above culture suspension was added in each serially diluted tubes followed by incubation for 24 hours and were observed for turbidity.

The interpretation were categorized as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI).

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To determine Time Kill, 3 different concentrations of triple antibiotic and Chlorhexidine Gluconate solution added to broth containing *E. Faecalis*and *C. Albicans* for 1min and 3min duration, and then broth smeared on agar plate and incubated for 24hrs. The microbial growth was analyzed in Colony forming units (CFUs).

Similarly time kill was checked at 1min and 3min for TAS and CHX on *E. Faecalis* and *C. Albicans*.

STATISTICAL ANALYSIS

Descriptive and inferential statistical analyses were carried out for the present study using software IBM SPSS statistics 21.0 (IBM Corporation, Armonk, NY, USA). Results on continuous measurements were presented as Mean ranks and results on categorical measurements were presented in Number (%). Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Based on the results of normality test (Kolmogorov smirnov& Shapiro wilk test), it was concluded that the data is not following the normal distribution, hence non parametric test were used. Kruskal Wallis test was used to find the significance of study parameters between three or more groups.

RESULTS:

Mean zone of inhibition of *E. Faecalis* by 50 mg, 100 mg and 150 mg of triple antibiotic was 40 mm. While by Chlorhexidine Gluconate mean zone of inhibition on *E. Faecalis* was 29.3 mm by 1% concentration, 29.7mm by 1.5% concentration and 35 mm by 2% concentration.

Mean Zone of inhibition of *C. Albicans* by triple antibiotic with 50 mg concentration was 2.3mm, of 100 mg was 12.3 mm and by 150 mg it was 16.3 mm. And Chlorhexidine mean zone of inhibition of 20.7mm by 1% concentration, 21.7mm by 1.5% concentration and 22.7mm by 2% conc. (Table 1).

By Kruskal Wallis Test:

There was statistically no significant difference in Zone Of Inhibition for three concentrations of triple antibiotic paste as well as Chlorhexidine Gluconate on *E. Faecalis*, while significant difference was noted on *C. Albicans*. (Table 2).

When pairwise comparison of Zone Of Inhibition was done, there was statistically significant difference among all the groups on E. Faecalis for all concentrations, except for 1% Chlorhexidine and 1.5% Chlorhexidine which was not statistically significant (p<0.05) (Table 3).

When pairwise comparison of Zone Of Inhibition was done on *C. Albicans*, the groups 1% Chlorhexidine Gluconate and 1.5% Chlorhexidine Gluconate, 1.5% Chlorhexidine Gluconate and 2% Chlorhexidine Gluconate showed statistically no significant difference. However for other statistically significant difference was seen. (Table 4).

Table 1: Descriptive findings of Zone of Inhibition (mm) on *E. Faecalis* (n = 3) and *C.Albicans*. (n = 3)

Microorganism	Group	Mean +SD
E. Faecalis	50 mg Triple antibiotic	40.0+.00
	100 mg Triple antibiotic	40.0+.00
	150 mg Triple antibiotic	40.0+.00
	1% Chlorhexidine	29.3+1.15
	1.5% Chlorhexidine	29.7+.58
	2% Chlorhexidine	35.0+1.00
C. Albicans	50 mg Triple antibiotic	2.3+.58
	100 mg Triple antibiotic	12.3+.58
	150 mg Triple antibiotic	16.3+.58
	1% Chlorhexidine	20.7+.58
	1.5% Chlorhexidine	21.7+.58
	2% Chlorhexidine	22.7+.58

Table 2: Comparison of zone of inhibition (mm) in between 3 concentrations of triple antibitotics on *E. Faecalis* and *C. Albicans*.

	Triple antibiotic solution		Chlorhexidine Gluconate	
	E. Faecalis	*C. Albicans*	*E. Faecalis*	*C. Albicans*
Chi-Square	.000	7.385	5.915	6.150
Df	2	2	2	2
p value	1.000	.025	.052	.046

Table 3: Pair wise Comparisons in between the 6 ingredients on the E. Faecalis zone of inhibition (mm).

Test Microorganism	(I) GROUP	(J) GROUP	Mean Difference (I-J)	p value
E. Faecalis	50 mg Antibiotic	1% Chlorhexidine	10.67	<0.001
	50 mg Antibiotic	1.5% Chlorhexidine	10.33	<0.001
	50 mg Antibiotic	2% Chlorhexidine	5.00	<0.001
	100 mg Antibiotic	1% Chlorhexidine	10.67	<0.001
	100 mg Antibiotic	1.5% Chlorhexidine	10.33	<0.001
	100 mg Antibiotic	2% Chlorhexidine	5.00	<0.001
	150 mg Antibiotic	1% Chlorhexidine	10.67	<0.001
	150 mg Antibiotic	1.5% Chlorhexidine	10.33	<0.001
	150 mg Antibiotic	2% Chlorhexidine	5.00	<0.001
	1% Chlorhexidine	1.5% Chlorhexidine	-.3	>0.05
	1% Chlorhexidine	2% Chlorhexidine	-5.7	<0.01
	1.5% Chlorhexidine	2% Chlorhexidine	-5.3	<0.01

* The mean difference is significant at the .05 level.

Table 4: Pair wise Comparisons in between the 6 ingredients on the C. Albicans zone of inhibition (mm).

Test Microorganism	(I) GROUP	(J) GROUP	Mean Difference (I-J)	p value
C. Albicans	50 mg Antibiotic	100 mg Antibiotic	-10.00	<0.001
	50 mg Antibiotic	150 mg Antibiotic	-14.00	<0.001
	50 mg Antibiotic	1% Chlorhexidine	-18.33	<0.001
	50 mg Antibiotic	1.5% Chlorhexidine	-19.33	<0.001
	50 mg Antibiotic	2% Chlorhexidine	-20.33	<0.001
	100 mg Antibiotic	150 mg Antibiotic	-4.00	<0.001
	100 mg Antibiotic	1% Chlorhexidine	-8.33	<0.001
	100 mg Antibiotic	1.5% Chlorhexidine	-9.33	<0.001
	100 mg Antibiotic	2% Chlorhexidine	-10.33	<0.001
	150 mg Antibiotic	1% Chlorhexidine	-4.33	<0.001
	150 mg Antibiotic	1.5% Chlorhexidine	-5.33	<0.001
	150 mg Antibiotic	2% Chlorhexidine	-6.33	<0.001
	1% Chlorhexidine	1.5% Chlorhexidine	-1.00	>0.05
	1% Chlorhexidine	2% Chlorhexidine	-2.00	<0.05
	1.5% Chlorhexidine	2% Chlorhexidine	-1.00	>0.05

* The mean difference is significant at the .05 level.

Table No. 5: Comparison of Time kill in between 3 concentrations of triple antibiotic and chlorhexidine on *E. Faecalis* and *C. Albicans* at 1min and 3min interval

Time Kill 1 min interval	Triple antibiotic	**150**	**100**	**50**
	E. Faecalis	NG	NG	NG
	C. Albicans	160 (CFU)	218 (CFU)	269 (CFU)
	CHX	**1%**	**1.5%**	**2%**
	E. Faecalis	NG	NG	NG
	C. Albicans	NG	NG	NG
Time Kill 3 min interval	Triple Antibiotic	**150**	**100**	**50**
	E. Faecalis	NG	NG	NG
	C. Albicans	48 (CFU)	100 (CFU)	122 (CFU)

	CHX	**1%**	**1.5%**	**2%**
	E. Faecalis	NG	NG	NG
	C. Albicans	NG	NG	NG

Table No. 6: comparison of MIC of triple antibiotic and Chlorhexidine Gluconate on *E. Faecalis* and *C. Albicans.*

E. Faecalis	**100**	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.6**	**0.8**	**0.4**	**0.2**
150	S	S	S	S	S	S	S	S	S	S
100	S	S	S	S	S	S	S	S	S	S
50	S	S	S	S	S	S	S	S	S	S
CHX										
1%	S	S	S	S	S	S	S	S	S	S
1.5%	S	S	S	S	S	S	S	S	S	S
2%	S	S	S	S	S	S	S	S	S	S
C. Albicans										
150	S	S	S	S	S	S	S	S	S	S
100	S	S	S	S	S	S	S	S	S	S
50	S	S	S	S	S	S	S	S	S	S
CHX										
1%	S	S	S	S	S	S	S	S	S	S
1.5%	S	S	S	S	S	S	S	S	S	S
2%	S	S	S	S	S	S	S	S	S	S

Note: NG-No Growth, S-Sensitive, R-Resistant

DISCUSSION

E. Faecalis and *C. Albicans* have been repeatedly identified as the most common species from root canals undergoing retreatment.*E. Faecalis* is gram positive cocci, facultative anaerobes with peptidoglycan and lipoteichoic acid component on cell wall which induces host immune response thus, capable of causing tissue destruction.

Yeasts, mainly *C. Albicans* proficiently invade dentinal tubules and are pathogenic even in small amounts.These microorganisms are distinct as they live and persist in poor nutrient environment of deeper layers of dentine[11]and survive even in the presence of several medication (e.g., calcium hydroxide), irrigants (e.g., sodium hypochlorite) and acquire antibiotic resistance.

The use of antibiotic in endodontics was first reported in 1951 by Grossman which was known as polyantibiotic paste (PBSC). Recently, the concept of revascularization of necrotic pulps has regained interest and became an alternative conservative treatment option for young permanent teeth with immature roots. "Lesion sterilization and tissue repair (LSTR) therapy" employs the use of a combination of antibacterial drugs (metronidazole, ciprofloxacin, and minocycline) for the disinfection of oral infectious lesions, including dentinal, pulpal, and periradicular lesions.[12,13].

Metronidazole exhibits broad spectrum of activity against protozoa and anaerobic bacteria. Minocycline also has broad spectrum of activity against gram positive and gram negative microorganisms. Ciprofloxacin is a synthetic floroquinolone with rapid bactericidal action.

Most of the previous studies have been done on triple antibiotic paste with or without vehicle/carrier however, none of the studies have been conducted on triple antibiotic mixture as an irrigant.

CHX is considered as gold standard of oral antiseptics, due to its broad spectrum antimicrobial and substantivity properties. It is recommended in gel or liquid form to disinfect root canals.Thus, the present in vitro study was designed to compare the antibacterial efficacy of triple antibiotic and chlorhexidine solution by employing well diffusion method followed by MIC and Time kill cure against *E. Faecalis and C. Albicans.*

Zone of Inhibition (ZOI)

The well diffusion method was used for the present study which is useful for evaluating and comparing the in vitro antimicrobial activities of medicaments before performing more advance test; many studies have used this method for evaluations of antibacterial effects of various endodontic materials.[14,15,16]

In the present study triple antibiotic solution measured zone of inhibition >40mm on pure culture of *E. Faecalis* on agar plate. The bacterial growths were almost negligible and were eliminated. Similar findings were reported in previousstudies[17-21] conducted by and also effective in sterilizing root canal and promoting healing of periapical pathology.

CHX was effective against *E. Faecalis* measured 32.5mm ZOI. The results were similar to thein-vitro studies conducted by Estrela et al[22], Lynne et al[23] and Ballal et al[24].Similarly, various studies[25-28] have shown that 2% Chlrohexidine gel or liquid form to reduce or completely eliminate E. Faecalisfrom the root canal space and dentinal tubules.

According to the present results, the TAS is more effective against *E. Faecalis* compared to CHX. This finding confirms with various studies using triple antibiotic mixture.[7,18,29,30,31]

In the present study triple antibiotic solution eliminates *E. Faecalis*even in minimum concentration (50mg each) that can be used for sterilization of root canals of primary teeth in children. Similar findings were reported by Sato et al[18]on *E. Faecalis*with this drug combination.

This lesser concentration can be used in a gentle treatment regimen (minimal or no instrumentation and an intracanal medication with TAP) before ERP which may conserve any viable tissue that may remain in the canal.[32]Many clinical investigations have reported an increase in root thickness and length, resembling normal maturation of the root after TAP therapy.[33-35]

Raison Bose compared TAP, calcium hydroxide, and formocresol as intracanal medicaments in non-vital young permanent tooth. The triple antibiotic group showed the highest percentage increase in the dentin wall thickness compared with the other two groups.[35]

The findings reported by de Lucena showed that the most effective medicament against *E. Faecalis* was TAP and after that in descending order were CHX, CH, NS and normal saline.[11]

In the present study CHX was more effective on *C. Albicans* measuring around 22mm ZOI compared to TAS of 150mg each concentration measuring 16mm ZOI. The results were comparable with In-vivo and in-vitro studies[1,24,36] and the effectiveness increased when combined with chitosan/propolis. This might be due to, the bactericidal action substantivity properties help to retainin close proximity to the canal walls and dentinal tubules.

MIC

The minimum inhibitory (MIC) concentrations TAS and CHX-digluconate were determined. Their results revealed that TAS and CHX-digluconate at different concentrations were effective against *E. Faecalis*and *C. Albicans*even when significantly diluted.

This is in accordance with the study conducted by Sabrah AH et al for TAP, the MIC and MBIC values were 0.003 mg/mL for *E. Faecalis*and decreased bacterial biofilm formation significantly.[37]

This suggests that TA Mixture diluted solution might be further used as efficient antibacterial irrigant during endodontic regeneration. 3 – Lenzi 2012.[38]

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In an experimental study on dogs[39], the intracanal drug delivery of a 20mg/ml solution of TAP resulted in >99% reduction in mean CFU levels.

Several in-vitro studies[40-42] using a broth dilution test have shown that 2% CHX is effective against *E. Faecalis*and other tested microorganisms in endodontics.[43]

In the study by Dametto et al, 2% CHX gel or liquid significantly reduced the number of *E. Faecalis*colonies.[44]

Similarly study conducted by Adl et al, showed the most effective medicament against E. Faecaliswas triple antibiotic powder/normal saline, with a MIC equal to 77.5ugram per mL, and on contrary 2% CHX was least effective against *E. Faecalis* with a MIC equal to 19500 µgm per mL.[7]

The antibiotic combinations were observed to be effective against both carious and endodontic lesions in-vitro. Hoshino et al determined that 25 µg each/ml of Ciprofloxacin, Minocycline and Metronidazole antibiotic mixture to be effective in sterilizing the infected root dentin in vitro.[32]

In other in vitro study the minimal inhibitory combination for 3mix (100ug each/ml) inhibited the growth of every strain completely.[45]

Ferguson et al sought to determine the in-vitrosusceptibility of *C.Albicans* to various irrigants and medicaments. The minimum inhibitory concentrations revealed that NaOCl, hydrogen peroxide and CHX-digluconate were effective against *C. Albicans* even when significantly diluted and study concluded that CHX is an effective antifungal agent, but its efficacy is significantly less than NaOCl.[46]
Kill curves with the advantage of providing more detailed information about the time course of antibacterial effect.

Time Kill
In time kill curve procedure after 1min and 3min duration, CHX showed no growth on tested organisms where as TAS showed no growth for *E. Faecalis*, but growth were seen for *C. Albicans*.

Similarly Chua et al showed the median reduction in CFU for *C. Albicans* was significantly higher in CHX group compared to TAP.[1]
Onça et al,[47] evaluated the anti microbial properties of 2% CHX for 5min and 48hr in extracted human teeth after the canals have been infected by *E. Faecalis* and *C. Albicans.*Both the 2% gels and 2% liquid formulations of CHX eliminated *C. Albicans*within 15s, whereas the gel formulation killed *E. Faecalis*within 1min.

Aqueous CHX solution has a wide spectrum of anti microbial activity at low concentrations and is especially effective against *C. Albicans,* which showed complete killing after 5mins.

The growths of *C.Albicans* after 1min and 3min could be attributed tachyplylaxis action of TA solution where there is rapid dissolution and reduced response to a drug.

CONCLUSION
The present study has its own limitations.The triple antibiotic solution as an irrigantwas most effective against *E. Faecalis*in comparison with chlorhexidine irrigating solution. It could eliminate *E. Faecalis*at low concentration so that it could be used in primary teeth of children. Chlorhexidine Gluconate was effective against *C. Albicans* in comparison with triple antibiotic mixture. Further in vivo and long term studies are recommended for use of triple antibiotic as a root canal irrigating solution.

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