

Effectiveness of a New Generation Anticavity Mouthwash on *Streptococcus mutans* and *Lactobacillus acidophilus* Count: A Microbiological Study

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ABSTRACT

Background Dental caries is one of the most widespread disease. CariFree CTx4 treatment rinse mouthwash is designed to treat the cavity-causing plaque biofilm, reduce the overpopulation of cavity-causing bacteria, and neutralize decay-causing acids with patent-pending pH⁺ technology.

Aim: The aim of the present study was to assess and compare the antibacterial effect of a new generation anticavity mouthwash (CariFree CTx4 treatment rinse) with that of a 0.2% chlorhexidine mouthwash (Clohex), 0.05% sodium fluoride mouthwash (Colgate Plax Complete Care), 2% povidone iodine mouthwash (Betadine gargle and mouthwash) against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and methods: Strains of *S. mutans* and *L. acidophilus* were grown on suitable media and the inhibitory effect of the test substances was noted by disk diffusion method and Agar well diffusion method. The results obtained were then subjected to statistical analysis.

Result: Efficacy of CTx4 treatment rinse mouthwash was less than chlorhexidine for both *S. mutans* and *L. acidophilus* whereas its efficacy was comparable to that of povidone iodine and sodium fluoride mouthwash for *S. mutans* and to that of sodium fluoride for *L. acidophilus*.

Conclusion: It was concluded that chlorhexidine is still the best mouthwash available.

Keywords: Anticavity mouthwash, CariFree CTx4 treatment rinse mouthwash, Chlorhexidine mouthwash, Sodium fluoride mouthwash, Povidone iodine mouthwash, *S. mutans*, *L. acidophilus*.

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INTRODUCTION

The tooth surface is unique in that it is the only body part that is not subjected to metabolic turnover. It is however subjected to various infections due to factors that favor microbial growth.¹ This microbial growth leads to one of the most widespread diseases of tooth, i.e. dental caries. *Streptococcus* bacteria are mainly responsible for the initial phase of the caries lesion especially in the enamel (initiation), whereas *Lactobacillus* is more involved with the progression of caries.² Targeting *Streptococcus mutans* forms the most important measure for prevention of dental caries.³ Current methods of caries management are limited to traditional preventive approaches in combination with restorative treatments have proved inadequate to control the disease. New methods of managing dental decay in the primary dentition need to be developed.⁴

This can be achieved by various mechanical and chemical aids. Many chemical antiplaque agents in the form of varnishes, dentifrices and mouthwashes have been tried for improvement of oral health. Mouthwashes have been found to be one of the safe and effective delivery system as antimicrobial and antiplaque agent. Among the various mouthwashes, the most persistent reduction of *S. mutans* has been achieved by chlorhexidine mouthwashes.³ However, it is not recommended for long-term use due to its numerous adverse effects like tooth and restoration staining, soft tissue staining, increased calculus deposition, unpleasant taste, taste alteration, burning sensation, desquamation and mucosal irritation.⁵ Fluoride mouthwash, commonly used in children is the sodium fluoride mouthwash. Some recently done studies demonstrated that fluoride mouthwash has potential antimicrobial activities.³ Povidone-iodine mouthwash has been shown to be effective in reducing plaque and gingivitis and may be a useful adjunct to routine oral hygiene.⁵ Newer mouthwashes which can be used for preventing dental caries are being introduced daily, one such mouthwash is CTx4 treatment rinse.

The aim of the present study was to assess and compare the antibacterial effect of a new generation anticavity mouthwash (CariFree CTx4 treatment rinse) with that of a 0.2% chlorhexidine mouthwash (Clohex), 0.05% sodium fluoride mouthwash (Colgate Plax complete care), 2% w/v povidone

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iodine mouthwash (Betadine gargle and mouthwash) and a control group (Distilled water) against *S. mutans* and *L. acidophilus*.

MATERIALS AND METHODS

Strains of *S. mutans* (MTCC no. 497) and *L. acidophilus* (MTCC no. 10307) were commercially obtained (Microbial Type Culture Collection Center, Chandigarh). For *S. mutans*, mitis salivarius bacitracin agar and for *L. acidophilus*, rogosa agar was commercially obtained from HIMEDIA. Microorganisms were activated 24 hours prior to the beginning of the study to obtain a suspension of 2×10^6 colony forming units/ml.

The Mouthwashes; CariFree CTx4 Treatment Rinse Mouthwash™, Colgate Plax Complete Care™, Betadine Gargle and Mouthwash™, Clohex Mouthwash™ were commercially obtained from the local market. The composition of these mouthwashes is given in Table 1.

0.1 ml of the test solutions were used in undiluted form for this antibacterial assay. Distilled water was used as control group. Antibacterial efficacy was tested using agar well diffusion and disk diffusion method. For Agar well diffusion method, 10 mm diameter wells were made in the medium with the help of a sterile steel borer and filled with 0.1 ml of the stock solution of mouthwashes. *S. Mutans* and *L. acidophilus* were incubated at 37°C, 48 hours, under aerobic conditions; two replicates were maintained for each mouthwash.⁸ For disk diffusion method disks made of the absorbent paper, with 5 mm diameter, obtained by patterned perforation of coffee filter paper were directly placed on the agar surface. These disks were properly sterilized and moistened in each test substance, as known: CariFree CTx4

Treatment Rinse mouthwash, Colgate Plax Complete Care, Betadine Gargle and Mouthwash, Clohex Mouthwash, Distilled Water. All of disks were immersed in equal time (superior to 1 min) in respective substances and in sequence, deposited neatly on sterile gauze to remove excess liquid. At the end of this phase, the dishes were transferred to incubator at 37°C for 48 hours.⁹ The antibacterial activity was measured as size of zone of inhibition (in millimeter). The results obtained were then statistically analyzed using one way ANOVA followed by Tukey HSD Test.

RESULTS

Streptococcus mutans

Cup Agar Method

Chlorhexidine showed greater diameter of inhibition zone followed by povidone iodine mouthwash, sodium fluoride mouthwash and CTx4 treatment rinse mouthwash in decreasing order (Figs 1 and 3).

According to Tukey HSD test, chlorhexidine showed statistically significant inhibition against *S. mutans* as compares to CTx4 treatment rinse mouthwash and sodium fluoride mouthwash. Betadine showed statistically significant inhibition as compared to CTx4 treatment rinse mouthwash (Table 2).

Disk Diffusion Method

Chlorhexidine showed greater mean diameter of inhibition zone against *S. mutans* followed by povidone iodine mouthwash, sodium fluoride mouthwash and CTx4 treatment rinse mouthwash (Figs 1 and 4).

Table 1: Name of the mouthwash used and their composition

Commercial name	Composition
Clohex	Chlorhexidine gluconate
Colgate plax complete care	Sodium fluoride, water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, cetyl peridinium chloride, sodium saccharin, menthol, methyl paraben, poly paraben, CI 42051
Betadine gargle and mouthwash	Povidone iodine
CariFree CTx4 treatment rinse	Rinse A: Active ingredient: Sodium fluoride (when mixed with rinse B), Inactive ingredients: Menthol, polysorbate 20, sodium benzoate, water, xylitol, natural flavors Rinse B: Inactive ingredients: sodium hydroxide, sodium hypochlorite, water

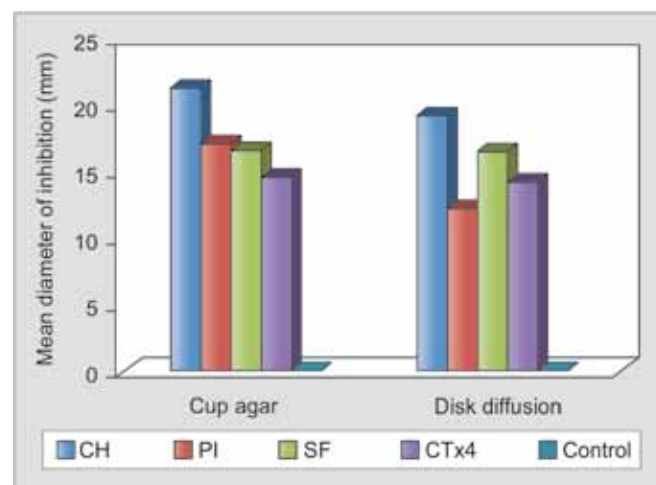


Fig. 1: Mean diameter of inhibition zone of test substances against *Streptococcus mutans* via both agar well diffusion as well as disk diffusion method (CH: Chlorhexidine; PI: Povidone iodine; SF: Sodium fluoride; CTx4: CTx4 treatment rinse mouthwash; Control: Normal saline)

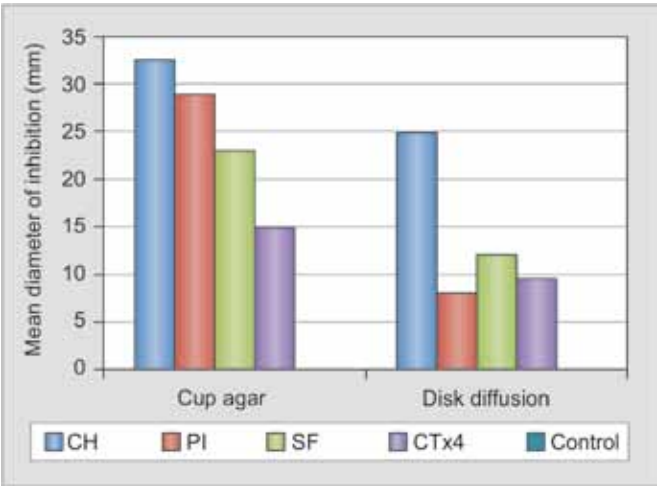


Fig. 2: Mean diameter of inhibition zone of test substances against *Lactobacillus acidophilus* via both agar well diffusion as well as disk diffusion method (CH: Chlorhexidine; PI: Povidone iodine; SF: Sodium fluoride; CTx4: CTx4 treatment rinse mouthwash; Control: Normal saline)

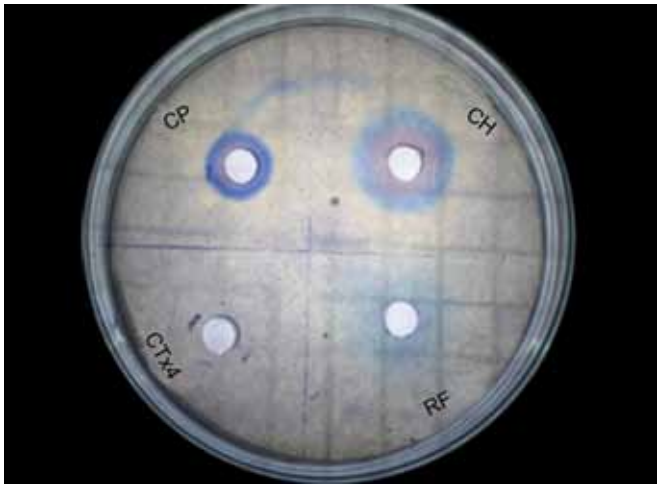


Fig. 3: Antimicrobial activity of test substances against *Streptococcus mutans* exhibited via agar well diffusion method

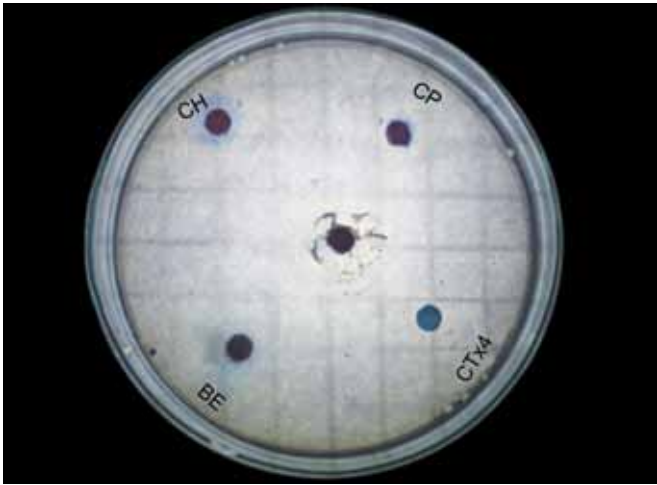


Fig. 4: Antimicrobial activity of test substances against *Streptococcus mutans* exhibited via disk diffusion method

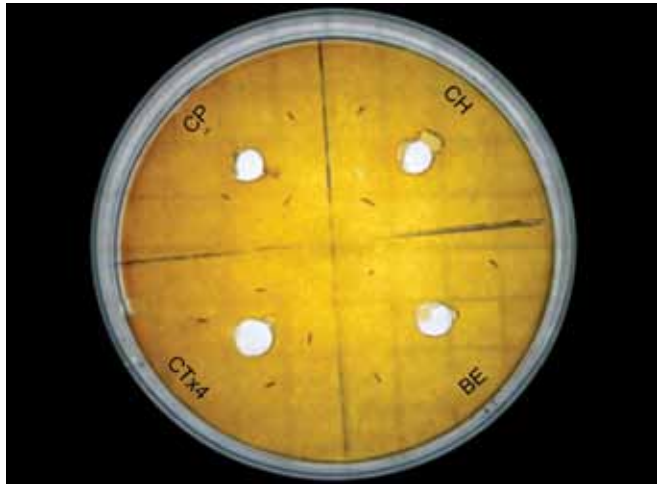


Fig. 5: Antimicrobial activity of test substances against *Lactobacillus acidophilus* exhibited via agar well diffusion method

According to Tukey HSD test, chlorhexidine showed statistically significant inhibition against *S. mutans* as compared to CTx4 treatment rinse mouthwash, sodium fluoride mouthwash and povidone iodine mouthwash (Table 3).

Lactobacillus acidophilus

Cup Agar Method

Chlorhexidine showed greater diameter of inhibition zone followed by sodium fluoride mouthwash, povidone iodine mouthwash and CTx4 treatment rinse mouthwash in decreasing order (Figs 2 and 5).

According to Tukey HSD test, no statistically significant difference in inhibition was observed between the mouthwashes used (Table 4).

Table 2: Tukey honest significant difference (HSD) test for *Streptococcus mutans* via cup agar method

Dependent variable	Group (I)	Group (J)	p-value
Cup agar	CH	BE	0.482
	CH	CTx4	0.156
	CH	CP	0.395
	BE	CTx4	0.807
	BE	CP	0.998
	CTx4	CP	0.887

CH: Clohex mouthwash; BE: Betadine gargle and mouthwash; CP: Colgate plax complete care mouthwash; CTx4: CariFree treatment rinse mouthwash

Disk Diffusion Method

Chlorhexidine showed greater mean diameter of inhibition zone against *S. mutans* followed by sodium fluoride mouthwash, povidone iodine mouthwash and CTx4 treatment rinse mouthwash in decreasing order (Figs 2 and 6).



Table 3: Tukey honest significant difference (HSD) test for *Streptococcus mutans* via risk diffusion method

Dependent variable	Group (I)	Group (J)	p-value
Disk diffusion	CH	BE	0.001
	CH	CTx4	0.004
	CH	CP	0.031
	CTx4	BE	0.086
	CP	BE	0.007
	CP	CTx4	0.060

CH: Clohex mouthwash; BE: Betadine gargle and mouthwash; CP: Colgate plax complete care mouthwash; CTx4: CariFree treatment rinse mouthwash

Table 4: Tukey honest significant difference (HSD) test for *Lactobacillus acidophilus* via cup agar method

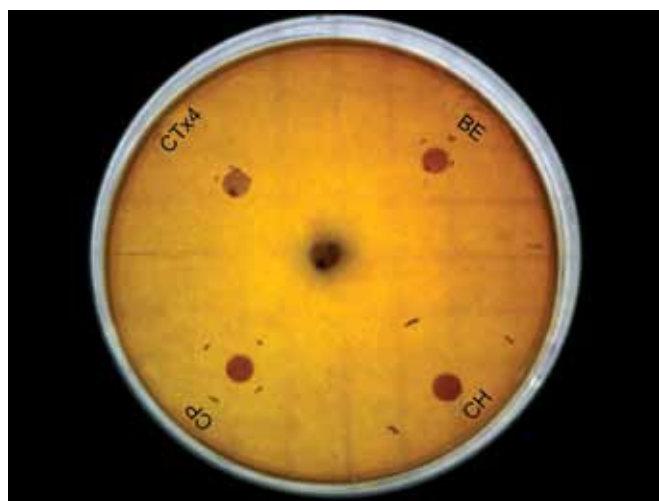
Dependent variable	Group (I)	Group (J)	p-value
Cup agar	CH	BE	0.537
	CH	CTx4	0.001
	CH	CP	0.032
	BE	CTx4	0.004
	BE	CP	0.226
	CTx4	CP	0.076

CH: Clohex mouthwash; BE: Betadine gargle and mouthwash; CP: Colgate plax complete care mouthwash; CTx4: CariFree treatment rinse mouthwash

Table 5: Tukey honest significant difference (HSD) test for *Lactobacillus acidophilus* via disk diffusion method

Dependent variable	Group (I)	Group (J)	p-value
Disk diffusion	CH	BE	0.010
	CH	CTx4	0.014
	CH	CP	0.027
	BE	CTx4	0.931
	BE	CP	0.444
	CTx4	CP	0.722

CH: Clohex mouthwash; BE: Betadine gargle and mouthwash; CP: Colgate plax complete care mouthwash; CTx4: CariFree treatment rinse mouthwash

**Fig. 6:** Antimicrobial activity of test substances against *Lactobacillus acidophilus* exhibited via disk diffusion method

According to Tukey HSD test, chlorhexidine showed statistically significant inhibition against *L. acidophilus* as compared to CTx4 treatment rinse mouthwash, sodium fluoride mouthwash and povidone iodine mouthwash. Also, sodium fluoride mouthwash showed statistically significant inhibition against *L. acidophilus* as compared to povidone iodine mouthwash (Table 5).

DISCUSSION

Among the various mouthwashes, chlorhexidine mouthwash is most widely used. The mechanism of action of a chlorhexidine mouthwash seems to be an immediate and probably short lived bactericidal effect, followed by a prolonged bacteriostatic action that is dependent on antiseptic absorbed by the pellicle coating tooth surface.⁶ In the present study, chlorhexidine was found to be more effective against both *S. mutans* and *L. acidophilus* via both agar well diffusion and disk diffusion method as compares to other mouthwashes studied. In previous studies also chlorhexidine was found to be more effective as compared to the other test substances used.⁷⁻¹⁰

Sodium fluoride mouthwash is most commonly used in children. This mouthwash is recognized as a potent anti-cariogenic agent and is effective in reduction of caries.³ Studies done earlier showed that sodium fluoride mouthwash showed no added advantage than chlorhexidine and that chlorhexidine still shows the best antimicrobial activity.^{11,12} In the present study, also the efficacy of sodium fluoride mouthwash was not better than chlorhexidine mouthwash.

Povidone-iodine is a water-soluble combination of molecular iodine and the solubilizing agent polyvinylpyrrolidone. This iodophor has a bactericidal effect similar to that of pure iodine; is effective against most of the bacteria, including putative periodontal pathogens, fungi, mycobacteria, viruses, and protozoa; fails to initiate sensitivity reactions or allow the development of bacterial resistance; and allows for a slow release of iodine, which ensures the establishment of an optimal, nontoxic concentration at a bactericidal level.⁶ Previous studies have showed that povidone iodine, as a mouthwash exerts only an immediate antibacterial effect and unlike chlorhexidine, is not retained at antibacterial levels within the oral cavity after expectoration.¹³

In the present study, CTx4 treatment rinse mouthwash, which contains sodium fluoride as active ingredient and xylitol, sodium hypochlorite and sodium benzoate as inactive ingredients, is used. 0.2% sodium hypochlorite oral rinse is bactericidal to all bacteria on contact. The FDA considers oral rinse solutions with less than 0.3% concentration of sodium hypochlorite safe for daily use. Though, it was

thought to be more effective than chlorhexidine, sodium fluoride and povidone iodine mouthwash; in the present study, efficacy of CTx4 treatment rinse mouthwash was found to be less than chlorhexidine on both test microbes, however, its efficacy was comparable to that of povidone iodine and sodium fluoride mouthwash for *S. mutans* and to that of sodium fluoride for *L. acidophilus*.

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