Efficacy of commercially available chlorhexidine mouthrinses against specific oral microflora

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ABSTRACT

Context: This study evaluates the antimicrobial efficacy of commercially available chlorhexidine (CHX) mouthrinses of different concentrations.

Aims: To evaluate and compare the antimicrobial efficacy of commercially available CHX mouthrinses of different concentrations (0.2%, 0.12%, and 0.1%) against specific standard strains of oral microflora at full strength (FS) and 1:1 dilution at 24 h.

Settings and Design: Ten commercially available 0.2% (Rexidine, Hexidine, Smilehex, Chlorhex, Hexidale, Hex, Everfresh, and Gargwell), 0.12% (Periogard), and 0.1% (Eludril) CHX mouthrinses were selected to evaluate the efficacy against specific oral microflora using agar well diffusion Method.

Materials and Methods: The standard strains of Streptococcus mutans American Type Culture Collection (ATCC 21293), Streptococcus sanguis Microbial Type Culture Collection (MTCC 442), Actinomyces viscosus (ATCC 3268), Staphylococcus aureus (ATCC 25923), Streptococcus pyogenes (MTCC 442), and Candida albicans (MTCC 183) were selected. The antimicrobial efficacy was calculated by measuring mean inhibitory zones formed on agar media.

Statistical Analysis Used: Independent t-test, one-way ANOVA, Kruskal–Wallis tests, and Tukey’s Post hoc analysis were used.

Results: Among 0.2% of CHX mouthrinses at FS and 1:1 dilution, hexidine was effective against most of the microorganisms except with S. pyogenes and C. albicans, where Hex and Hexidale were effective, respectively. When the concentration of 0.1% and 0.12% CHX was considered, Eludril was more effective at FS against all except with S. aureus and S. pyogenes which were more sensitive to Periogard at both FS and 1:1 dilution.

Conclusions: 0.12% and 0.1% of CHX mouthrinses showed comparable efficacy with 0.2% CHX mouthrinses irrespective of their formulations.

Key words: Chlorhexidine, dental plaque, mouthrinses, salivary bacteria

Dental plaque as defined by Allison et al. 20001 is the diverse communication of microorganisms found on tooth surface as a biofilm, embedded in an extracellular matrix of polymers of host and microbial origin.

The Gram-positive bacteria Streptococcus mutans are a substantial part of the oral microbiota and their importance in the dental caries etiology is unquestionable.2 The carbohydrates present in the diet are the main energy source in an anaerobic process (mainly lactic fermentation) resulting in the production of organic acids. These acids decrease the pH to around 4.5 on the tooth surface, thus inducing its demineralization.3 Streptococcus sanguis, a major plaque-forming organism4 to which Actinomyces viscosus adheres through surface fimbriae to polysaccharide receptor on cells of S. sanguis. These types of interactions are thought to be of primary importance in the colonization of the periodontal environment.5

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Among the microorganisms present in the oral cavity, the reduction in the number of *Staphylococcus aureus before* surgical procedures has been associated with a lower incidence of infective endocarditis and postoperative infections. On the other hand, the presence of large numbers of *Streptococcus pyogenes* in the oral cavity usually correlates with acute pharyngeal infection. *Candida albicans* also is the most common yeast isolated from the oral cavity and a common cause of oral thrush. These microorganisms were selected aiming to represent the wide variety of microbial species present in the dental biofilm.

The existence of these micro-organisms as the polypsies in the oral cavity has profound implications in the etiology of dental caries, periodontal, and other systemic diseases. Control of oral microbiota by antimicrobial mouthrinses as an adjunctive to mechanical plaque control has been considered to be an effective method of preventing dental diseases.

Mouthrinses have been used for centuries for medicinal and cosmetic purposes, but it is only in recent years that the rationale behind the use of their ingredients has been subjected to scientific research and clinical trials. Mouthrinses are solutions or liquids used to rinse the mouth for a number of purposes: (a) Prevent the biofilm formation (b) inhibition of early microbial colonization on tooth surfaces (c) the alteration of pathogenic plaque into nonpathogenic plaque, and (d) to have a therapeutic effect by relieving periodontal infections or preventing dental caries.

Evidence in dental literature support and recognize, chlorhexidine (CHX), as the gold standard against which other antiplaque and antigingivitis agents are measured. CHX, a cationic bisbiguanide biocide was first described in the 1940s by Imperial Chemical Industries, England. Later, Davis et al. in 1954 concluded that the agent with the greatest bacteriostatic and bactericidal features 1:6-Di-4’-chlorophenyldiguanidohexane, a synthetic cationic detergent usually referred as “Chlorhexidine.”

Plaque inhibition by CHX was first investigated by Schroeder in 1969, but the definite study was performed by Loe and Schiott in 1970. *In vitro* studies showed that, in low concentrations, CHX causes damage to the cell membrane, and low molecular weight molecules escape from the microorganisms. At higher concentrations, CHX causes precipitation and coagulation of the proteins in the cytoplasm of the exposed microbes. These properties interfere with biofilm formation and prevent the growth processes.

The long-term efficacy and safety of CHX mouthrinse have been proven in several studies. Regardless of potent antimicrobial and antiplaque properties of CHX, its widespread and comprehensive uses are restricted by local side effects which mostly dose dependent.

Thus, by understanding the properties and limitations of the CHX molecule, consideration has been given to CHX with lower concentrations of 0.12% (Periogard) and 0.1% (Eludril) which are commercially available. CHX mouthwashes exist not only at different concentrations but also in different formulations. Although CHX is an effective agent in different concentrations (0.2%, 0.12%, and 0.1%), administration of these antimicrobial agents requires a careful evaluation for clinical situation and an accurate analysis.

With this background, the purpose of the study was to evaluate and to compare the antimicrobial efficacy of ten commercial available CHX mouthrinses of different concentrations (0.2%, 0.12%, and 0.1%) against specific strains of oral microflora at full strength (FS) and 1:1 dilution at 24 h.

**MATERIALS AND METHODS**

Various concentrations of ten commercially available 0.2% (Rexidene, Hexidine, Smilehex, Chlorhex, Hexidale, Hex, Everfresh, and Gargwell), 0.12% (Periogard), and 0.1% (Eludril) CHX mouthrinses [Table 1] were selected to evaluate the efficacy against specific standard strains (American Type Culture Collection [ATCC] and Microbial Type Culture Collection [MTCC]) of oral microflora at FS and 1:1 dilution at 2 4 h. The selected standard strains were *S. mutans* (ATCC 21293), *S. sanguis* (MTCC 442), *Actinomyces viscosus* (ATCC 3268), *S. aureus* (ATCC 25923), *S. pyogenes* (MTCC 442), and *C. albicans* (MTCC 183). These microorganisms were subcultured on specific media and incubated aerobically at 37°C. The identification of all the microorganisms was confirmed by standard biochemical and microscopical analysis. Ethical clearance was obtained from the Institutional Review Board (PMVIDS/PHD/0011/2012).

The antimicrobial efficacy of selected mouthwashes was assessed using agar well diffusion method.

The mouthwashes were tested at FS (100%) and at 1:1 dilution (50%) by taking sterile distilled water as the diluent. Sterile distilled water was used as a control. A 25 µl volume of each mouthwash (at FS and at 1:1 dilution) and the control was dispensed directly into the wells (in triplicates) of the inoculated specific media plates for each test microorganisms. The plates allowed standing for 1 h for diffusion of mouthwash to take place and incubated at 37°C for 24 h. The antimicrobial activity was measured based on the diameter of inhibition zone formed around the well containing the mouthwash. The experiment was performed in triplicates, and mean values of the diameter of inhibition zones with standard deviation were calculated.
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RESULTS

Sterile distilled water (control) did not show any zones of inhibition against tested microorganisms.

Table 2 represents mean inhibitory zones of FS and 1:1 dilution at 24 h against specific oral microflora. Among 0.2% of CHX mouthrinses at FS and 1:1 dilution, Hexidine showed the highest mean inhibitory zones against most of the microorganisms except with S. pyogenes and C. albicans, where Hex and Hexidale produced larger inhibitory zones, respectively.

When the concentration of 0.1% (Eludril) and 0.12% (Periogard) CHX was considered, Eludril was more effective at FS against all except with S. aureus and S. pyogenes, which were more sensitive to Periogard at both FS and 1:1 dilution.

Within group comparison at FS and 1:1 dilution demonstrated that Eludril was significantly more effective at FS as compared to 1:1 dilution for all tested microorganisms. Hexidine (P = 0.01), Hex (P = 0.04), Everfresh (P = 0.00), and Gargwell (P = 0.01) inhibited S. sanguis growth to large extent at FS. Likewise, Everfresh (P = 0.006), Gargwell (P = 0.004), and Periogard (P = 0.02) inhibited A. viscosus growth and Chlorhex (P = 0.006), Smilehex (P = 0.02), and Hexidale (P = 0.003) inhibited S. pyogenes growth with significant effectiveness at FS.

Between-group comparisons revealed significant difference for all selected microorganisms.

Pair-wise comparison of mean inhibitory zones at FS and 1:1 dilution using Tukey’s post hoc analysis. Hexidine at FS and 1:1 dilution and Eludril at FS showed a significant difference with other mouthrinses against S. mutans, S. sanguis, and A. viscosus.

With regards to S. sanguis, Chlorhex, and Periogard equally showed significant difference with other study mouthrinses at 1:1 dilution. Concerning A. viscosus, at 1:1 dilution apart from Hexidine and Eludril, Gargwell, Everfresh, and Periogard also significantly differed with remaining mouthrinses except with each other. Hexidine, Hexidale, Periogard, and Chlorhex at FS and 1:1 dilution and Eludril only at 1:1 dilution showed significant difference against S. aureus, which exhibited equal sensitivity to Hexidine, Hexidale, and Periogard at FS.

Against S. pyogenes, at FS and 1:1 dilution, Hex exhibited significant difference. Furthermore, Rexidine, Hexidine, Eludril also equally exhibited significant difference with other mouthrinses for the same. At FS, Smilehex, Chlorhex, Hexidale, Everfresh, and Gargwell showed comparable effectiveness against S. pyogenes. At 1:1 dilution, Everfresh, Gargwell, Periogard, and Eludril significantly differed with other study mouthrinses. With respect to C. albicans, a significant difference was observed with Hexidale at FS and 1:1 dilution and Eludril only at FS.
TABLE 2: Mean Inhibitory zones of full strength (FS) and 1:1 dilution at 24 hrs against specific oral microflora

| Microorganisms     | Time  | Tested | Concentration | Controls | CHX | P-value |
|--------------------|-------|--------|---------------|----------|-----|---------|
|                    |       |        | 0.2% CHX      | FS       | HD  |        |
| S. mutans (ATCC 21299) | 24 hrs | FS     | 0.2% CHX      | 0.2% CHX | 0.2% CHX | 0.2% CHX |
|                    |       |        | 0.12% CHX     | 0.12% CHX | 0.12% CHX | 0.12% CHX |
|                    |       |        | 0.1% CHX      | 0.1% CHX | 0.1% CHX | 0.1% CHX |
|                    |       |        | FS            | FS       | FS  |        |
|                    |       |        | HD  |        |        |        |
| A. viscosus (MTCC 442) | 24 hrs | FS     | 0.2% CHX      | 0.2% CHX | 0.2% CHX | 0.2% CHX |
|                    |       |        | 0.12% CHX     | 0.12% CHX | 0.12% CHX | 0.12% CHX |
|                    |       |        | 0.1% CHX      | 0.1% CHX | 0.1% CHX | 0.1% CHX |
|                    |       |        | FS            | FS       | FS  |        |
|                    |       |        | HD  |        |        |        |
| S. pyogenes (MTCC 3140) | 24 hrs | FS     | 0.2% CHX      | 0.2% CHX | 0.2% CHX | 0.2% CHX |
|                    |       |        | 0.12% CHX     | 0.12% CHX | 0.12% CHX | 0.12% CHX |
|                    |       |        | 0.1% CHX      | 0.1% CHX | 0.1% CHX | 0.1% CHX |
|                    |       |        | FS            | FS       | FS  |        |
|                    |       |        | HD  |        |        |        |
| C. albicans (ATCC 90028) | 24 hrs | FS     | 0.2% CHX      | 0.2% CHX | 0.2% CHX | 0.2% CHX |
|                    |       |        | 0.12% CHX     | 0.12% CHX | 0.12% CHX | 0.12% CHX |
|                    |       |        | 0.1% CHX      | 0.1% CHX | 0.1% CHX | 0.1% CHX |
|                    |       |        | FS            | FS       | FS  |        |

**DISCUSSION**

The efficacy of the 0.2% CHX on plaque, gingivitis, microbial ecology, and side effects have been demonstrated in many studies,[19,20,26-28] and the product can be used as positive control to compare other formulations including those containing CHX in different concentrations. Changes in the concentration of CHX may, however, produce an impact on their activity and therefore, these concentrations need be evaluated in well-designed studies. Sterile distilled water used as negative control to obtain valid comparisons between study mouthrinses. The antibacterial effect of CHX mouthrinses was evaluated at 24 h to match routine oral hygiene practices.

In this study, agar well diffusion method was employed as it is a well-established technique commonly used in screening the antimicrobial efficacy of chemicals before in vivo testing.[23-29] This present study evaluated the antimicrobial efficacy at both FS and 1:1 dilution to avoid superfluous exposure to higher concentration if diluted form is equally effective. Furthermore, side effects also would be reduced which is dose dependent.[22]

In this study, a maximum number of mouthrinses was equally effective at FS and 1:1 dilution except Eludril, which was significantly more effective at FS than 1:1 dilution for all tested microorganisms. Likewise Hexidale, Hex, Everfresh, and Gargwell inhibited S. sanguis; Everfresh, Gargwell, and Periogard against A. viscosus and Chlorhex, Smilehex, and Hexidale against S. pyogenes exhibited significant effectiveness at FS.

The results of this in vitro investigation clearly showed that commercially available mouthrinses containing the same active ingredient (CHX) at different concentrations (0.2%, 0.12%, and 0.1%) demonstrated a significant difference in their antimicrobial activity. Out of the eight 0.2% CHX mouthrinses used in this study demonstrated that Hexidine was effective against majority of mouthrinses (S. mutans, S. sanguis A. viscosus, and S. aureus), whereas Hex and Hexidale produced larger inhibitory zones against S. pyogenes and C. albicans. The studies done by Emilson CG26 and Emilion et al.[26] evaluated the minimum inhibitory concentrations of 0.2% CHX for streptococcal species (S. mutans and S. sanguis) and also reported that S. mutans and S. sanguis were sensitive to CHX.

When the concentration of 0.1% (Eludril) and 0.12% (Periogard) CHX was considered, Eludril did not show a similar degree of activity with Periogard against dental caries causing microorganisms (S. mutans, S. sanguis, and A. viscosus) and also with C. albicans. These differences may be due to the difference in the CHX concentration and disparity with other unknown factors related to the whole formulations of these commercial products.
While correlating this in vitro study results with several in vivo studies, Bay\textsuperscript{[30]} concluded that the effects of 0.05% CHX were less effective than the 0.1%, and 0.15% CHX solutions on plaque and gingivitis. Briner and Leonard\textsuperscript{[31]} also conducted a dose–response study in beagle dogs and conveyed that 0.1% and 0.2% solutions produced a significant reduction in gingivitis than controls. Another study done by Lang et al.\textsuperscript{[32]} found no difference between 0.1% and 0.2% mouthrinses. On the other hand, Jenkins et al.\textsuperscript{[33]} reported that the plaque inhibitory effect of CHX and chlorhexidine in vitro. Further in vivo studies should be supported to distinguish the clinical efficacy of 0.1% chlorhexidine with (Eludril) and 0.2% chlorhexidine mouthrinses. J Clin Periodontal 1995;22:613-7.

CONCLUSIONS

The present study concluded that overall antimicrobial efficacy of selected mouthrinses showed good results against specific oral microflora. 0.12% and 0.1% of CHX mouthrinses showed comparable efficacy with 0.2% CHX mouthrinses irrespective of their formulations. How so ever further in vivo studies should be supported to distinguish the influence of dietary habits and oral hygiene practices on clinical efficacy of 0.1% chlorhexidine with (Eludril) and 0.12% (Periogard).

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Conflicts of interest

There are no conflicts of interest.

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