

In vitro antimicrobial comparison of three commercially available chlorhexidine-based oral rinses

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ABSTRACT

Introduction: Commercially available chlorhexidine (CHX) formulations differ in their CHX concentrations (0.2% and 0.12%) as well as in various additives including alcohol, antimicrobials such as cetylpyridinium chloride and anti-discolouration chemicals such as ascorbic acid and sodium metabisulphite.

Aims and objectives: To compare *in vitro* the antimicrobial efficacies of three different CHX preparations (Corsodyl[®], Curasept[®] and GUM[®] Paroex[®]) using 0.2% and 0.12% CHX concentrations as controls

Methods: A disk diffusion test was performed using pure cultures of the organisms *Streptococcus mutans* and *Candida albicans*, and mixed cultures (facultative and strict anaerobes) prepared from oral rinse samples of 14 study participants. The means and standard deviations of the diameters of inhibition zones were calculated.

Results: A statistically significant difference (p value = 0.0001) was found only in *Candida albicans* cultures between the mean inhibition zones of the CHX preparation disks. Pure CHX preparations and Corsodyl[®] showed higher antifungal efficacy than Curasept[®] and GUM[®] Paroex

Conclusion: Both CHX preparations (0.12% and 0.2%) and the 0.2% CHX preparation containing alcohol (Corsodyl[®]) have more potent antifungal properties against *C. albicans* than alcohol-free 0.12% CHX preparations such as Curasept[®] and GUM[®] Paroex[®].

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ACRONYMS

CHX:	chlorhexidine
CFU:	colony forming units
BHI:	Brain Heart Infusion

INTRODUCTION

Chlorhexidine (CHX) is the most commonly used antimicrobial agent in dentistry. Because of its wide range of antimicrobial activity, it has been incorporated as an antiplaque agent into several oral hygiene products such as dentifrices and mouthrinses. Based on its clinical efficacy, CHX is currently regarded as the “gold standard” for evaluating new chemical plaque control agents.¹

Most commercially available mouthrinses, including some CHX based mouthrinses, contain alcohol, with concentrations being as high as 14-15%.² However, the addition of alcohol is controversial because of its potential carcinogenic and tissue irritating properties. Several manufacturers have therefore developed alcohol-free CHX based oral hygiene products as alternatives.² Limited data exists with regards to the antibacterial efficacy of these products, with studies indicating that alcohol-based CHX formulations are more effective than alcohol-free formulations.²

Commercially available CHX formulations differ in their concentrations as well as in the component additives. Most CHX mouthrinses are prepared in concentrations of 0.2% or 0.12%. In this study, the antimicrobial efficacy of three locally available CHX based mouthrinses i.e. Corsodyl Mouthwash[®] 0.2% CHX (GlaxoSmithKline, Epping, South Africa), Curasept ADS[®] 220 Oral rinse 0.2 % CHX (Curaden AG, Krein, Switzerland) and GUM[®] Paroex[®] 0.12% CHX (Sunstar Europe S.A., Etoy, Switzerland) were evaluated for their antimicrobial efficacy.

MATERIALS AND METHODS

Study design

An *in vitro* analytical comparative study was carried out to evaluate three commercially available CHX-based mouthrinses.

Table 1: Inclusion and exclusion criteria used for recruitment of study participants.

Inclusion criteria
Dentate and partially dentate individuals
Adults > 12 years of age
Systemically healthy
Exclusion criteria
Edentulous individuals
Children > 12 years of age
Patients with systemic conditions
Smokers
Individuals who have used antibiotics or immunosuppressive drug therapies during the past three months
Persons with active periodontal disease
Persons with active carious lesions
Persons with clinically evident oral candidiasis

Study sample

Oral rinse samples were collected from 14 healthy staff members at the University of the Western Cape Dental Faculty (UWC) who met the inclusion criteria (Table 1).

Specimen preparation and data collection

Each subject was supplied with 10 ml of sterile saline in a universal container and instructed to rinse his/her mouth in the presence of the researcher for 60 seconds and then to return the mouth rinse to the container.⁶ 100µl of the rinse was inoculated onto previously prepared Brain Heart Infusion agar plates (BHI), by spreading the sample over the agar surface with a sterile glass rod. For each oral rinse sample, two plates were prepared, one for facultative anaerobic cultures, and the other for strictly anaerobic cultures. The latter was done to culture Gram negative anaerobic bacteria, such as *Veillonella* and *Fusobacteria*.⁷ The anaerobic conditions were created inside an anaerobic jar utilizing an Oxoid® Gas generating kit (UK), with palladium as a catalyst. A colour indicator was used to signal the transformation to an anaerobic environment. For the facultative anaerobic cultures, an anaerobic incubator was used. The incubation period for both culture types was 24 hours.

Preparation of pure cultures

Pure cultures of *S. mutans* NCTC 25175 and *C. albicans* NCTC 36801 were selected, as these microorganisms are known aetiological factors for dental caries and candidiasis respectively. These were cultured in the laboratory for 24 hours. Thereafter, a separate inoculum from each culture was prepared. This was done by selecting an appropriate

culture and preparing a suspension thereof in saline using the direct colony suspension method.

The two suspensions (*S. mutans* and *C. albicans*) were standardized to 0.5 McFarland standard (corresponding approximately to 1.5 X 10⁸ CFU/ml). The McFarland scale is used for measuring bacterial densities in suspensions. There was no need to standardize the turbidity of the oral rinse samples since its natural turbidity closely approximated that of the 0.5 McFarland standard.

100µl of each suspension was inoculated onto 14 standard BHI plates within a quarter of an hour of the suspension preparation. Sterile glass-rods were used to spread the suspension evenly over the surface of the plate. This produced an acceptable distribution of the bacterial colonies on the surface of the 28 agar plates.

The CHX preparations

Three commercially available mouthrinses, representing the most prevalent CHX-based rinses in South African markets, were purchased from local stores, whilst the controls - (only CHX formulations) were prepared by the Institute of Oral and Dental Research at the Faculty of Dentistry, University of the Western Cape (Table 2).

The control CHX formulations (referred to hereunder as "only CHX") were water based and alcohol free solutions that were prepared in two different concentrations i.e. 0.2% & 0.12%. The only CHX 0.2% acted as a control for Corsodyl® and Curasept ADS® 220 (both containing CHX 0.2% concentration), whilst only CHX 0.12% acted as the control for GUM® Paroex® which contained CHX 0.12%.

Disk Diffusion Test to measure inhibition zones:

The 56 agar plates used for the disk diffusion test were divided equally into four groups as listed below:

- Group 1:** 14 facultative anaerobically cultured plates prepared from oral rinse samples.
- Group 2:** 14 strict anaerobically cultured plates prepared from oral rinse samples.
- Group 3:** 14 plates of pure cultures of *S. mutans* bacteria.
- Group 4:** 14 plates of pure cultures of the fungus *C. albicans*.

The disk diffusion test was performed by adding five sterile, 6mm diameter filter paper disks to each of the 56 BHI plates. The disks were evenly distributed on the agar surface. Each disk was saturated with 10µl of a specific CHX

Table 2: Description of the Chlorhexidine (CHX) oral rinses used in the study

Codes as per disk diffusion test	CHX preparation	Active ingredients	Inactive ingredients
CHX 1	only CHX 0.2% (laboratory prepared)	CHX 0,2%	N/A
CHX 2	only CHX 0.12% (laboratory prepared)	CHX 0,12%	N/A
CHX 3	Corsodyl Mouthwash® (GlaxoSmith-Kline, Epping, South Africa)	CHX 0.2%, Ethanol 5-6%	Unassigned
CHX 4	Curasept ADS® 220 Oral rinse 0.20 % CHX (Curaden AG, Krein, Switzerland)	CHX 0.2%	ADS, Xylitol, propylene glycol, PEG 40, hyd. castor oil, ascorbic acid, Poloxamer 407, sodium metabisulfite sodium citrate, aroma Cl.42090
CHX 5	GUM® Paroex® (Sunstar Europe S.A., Etoy, Switzerland)	CHX 0.12%, Cetylpyridinium Chloride 0.05%	Aqua, propylene glycol, glycerine, PEG 40 hydrogenated castor oil, aroma, potassium acesulfame, methylparaben, propylparaben, C.I. 14720, limonene

product to be tested and an identifying code number assigned. The antibacterial effect of each CHX product was quantified in terms of the formation of inhibition zones around the disks, measured 24 hours following incubation (Figure 1).

All measurements were carried out using a digital calliper, by both the principal investigator and by a second clinician, who was blinded to the results obtained by the former. The diameter of each inhibition zone was measured thrice by each investigator. If a discrepancy of >1mm was found, the measurements were repeated. An average of the readings of each investigator was taken. Data capturing tables were used to record the readings.

Data analysis

The mean diameters and standard deviations of the corresponding inhibition zones were calculated and compared using the analysis of variance (ANOVA) test. A P value of less than 0.05% was considered statistically significant.



Figure 1: Typical agar plates showing *S. mutans* and *C. albicans* growth colonies (above and below respectively). BHI agar plate Number 5 (of 14) showing inhibition zones around different CHX preparations. Numbers on filter papers denotes the following: 1: CHX1 (only CHX 0.2%), 2: CHX2 (only CHX 0.12%), 3: CHX3 (Corsodyl® - 0.2% CHX), 4: CHX4 (Curasept® - 0.12%CHX) and 5: CHX5 (GUM® Paroex® - 0.12% CHX)

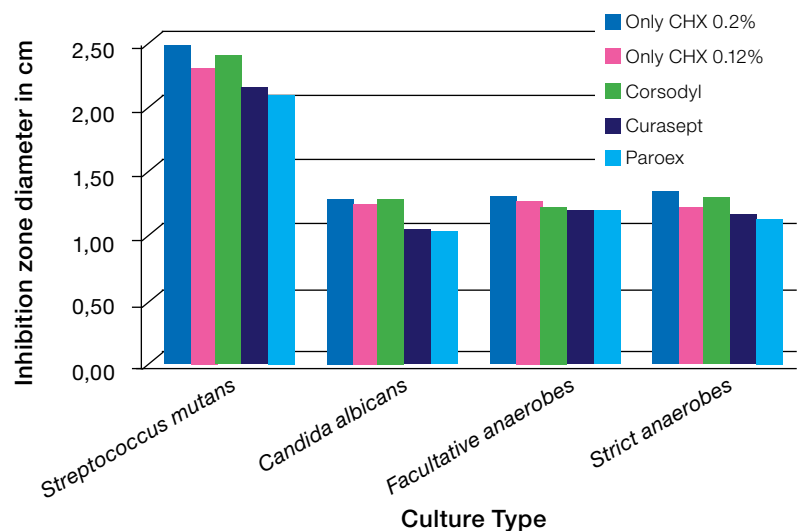


Figure 2: Graph comparing mean inhibition zones for chlorhexidine (CHX) preparations.

RESULTS

A statistically significant difference in the inhibition zones was found only in the fungal cultures, as analyzed by the ANOVA one-way test. Both Curasept® (0.12% CHX) and GUM® Paroex® (0.12% CHX) showed significantly smaller inhibition zones ($p < 0.05$) while the only CHX (0.2% and 0.12%) as well as Corsodyl® (0.2% CHX), produced inhibition zones of comparable sizes (Table 2). When the inhibition zones produced by the different cultures were compared, all the formulations gave significantly larger zones towards *S. mutans* than did any of the other cultures. No significant differences were recorded between the zones produced by facultative anaerobic and strict anaerobes from the mixed saliva cultures.

When the means of inhibition zones for all CHX formulations were considered together, readings could be represented as in Figure 2.

DISCUSSION

The results of the study showed no statistically significant differences between the antibacterial activities of the three mouthrinses evaluated (Corsodyl®, Curasept® and GUM® Paroex®), regardless of the CHX concentration or the brand of oral rinse tested. The antimicrobial efficacies of all CHX formulations were highest against *Streptococcus mutans*, when compared with other cultures, supporting the anti-cariogenic role of CHX and its use as an adjuvant to mechanical oral hygiene measures.⁸

Even though a statistically significant difference was found with regard to the antifungal activity between the three oral rinses, the inhibition zones produced for *C. albicans* cultures were smaller than those recorded for *S. mutans* cultures. Only CHX preparations and Corsodyl® showed a higher antifungal activity compared with Curasept® or GUM® Paroex®. This finding is consistent with the literature where *C. albicans* displayed a lower susceptibility to CHX. This is thought to be due to the greater complexity of the fungal cell membranes as compared with that of Gram positive bacteria.¹⁰

For both *S. mutans* and *C. albicans*, the readings for antimicrobial sensitivity across the 14 cultures were numerically closer than in both types of cultures prepared

Table 3: Group means and Standard Deviations for inhibition zones (in cm)

n=14	Only CHX 0.2%	Only CHX 0.12%	Corsodyl® 0,2%CHX	Curasept® 0.12% CHX	GUM® Paroex® 0.12% CHX
<i>Streptococcus mutans</i>	2.48± 0.37	2.31± 0.47	2.39± 0.44	2.15± 0.39	2.10± 0.32
<i>Candida albicans</i>	1.30± 0.09	1.25± 0.11	1.29± 0.11	1.06± 0.06	1.05± 0.10
Facultative anaerobes	1.31± 0.36	1.28± 0.36	1.23± 0.34	1.20± 0.34	1.19± 0.34
Strict anaerobes	1.34± 0.29	1.21± 0.29	1.29± 0.29	1.17± 0.27	1.13± 0.40
ANOVA p value ≤ 0.0001					

from participants' oral rinses. Such variability reflects the qualitative differences in oral microbial flora between individuals.

Only CHX (0.2%) exhibited the highest antimicrobial efficacy followed by Corsodyl® (containing 5% alcohol) and then Curasept®. The latter has a similar CHX concentration but is alcohol free. Alcohol acts as an emulsifier, as a solvent for active ingredients, as a preservative and as an antiseptic.¹³ Perhaps the alcohol in Corsodyl® acted as a diluent of CHX and the additive in Curasept® (anti-discolouration systems) could have decreased the antimicrobial efficacy of CHX⁴, due to interaction with the strong positive charge of the CHX molecule.⁹

Even though GUM® Paroex® contains 0.05% CPC, it was surpassed in antimicrobial efficacy by pure CHX 0.12%. This could probably also be attributed to the aforementioned chemical interactions between CHX and additives, thereby reducing its efficacy. Nevertheless, GUM® Paroex® had similar antimicrobial efficacy as Curasept® (0.2% CHX), and this was probably due to the fact that it contained 0.05% CPC.

CONCLUSION

The results of the study show that both the pure (0.12% and 0.2%) CHX preparations as well as the 0.2% CHX preparation containing alcohol (Corsodyl®) have more potent antifungal properties against *C. albicans* than does alcohol-free 0.12% CHX preparations such as Curasept® and GUM® Paroex®. CHX preparations are effective against most classes of oral microflora and is well chosen as an adjunct to plaque removal.

Ethical approval for the study was obtained from the UWC Dental Faculty.

Conflict of Interest: None declared.

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