The antimicrobial efficacy of three chlorhexidine mouth rinses: an *in-vitro* analysis

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for the degree of Master of Science in Dental Sciences in Periodontics & Oral Medicine at the
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The antimicrobial efficacy of three chlorhexidine mouth rinses: an *in-vitro* analysis

Key Words

Chlorhexidine

Antimicrobial

Efficacy

Dental Plaque

Oral rinse

Facultative anaerobes

Strict anaerobes

Streptococcus mutans

Candida albicans

Disk diffusion test

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ABSTRACT

Different Chlorhexidine (CHX) preparations and formulations are available in local markets. Some preparations contain Anti-discoloration systems (ADS), additional antimicrobials like Cetylpyridinium chloride (CPC), or alcohol. The aim of this study was to compare the antimicrobial efficacies of 3 different CHX preparations (Corsodyl®, Curasept® and GUM® Paroex®).

Methods:

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

Results:

ANOVA test was used to verify whether the differences in means were statistically significant. Accordingly, a statistically significant difference (p. value = 0.0001) was found only in *Candida albicans* cultures between the mean inhibition zones of the different CHX preparation disks. Pure CHX preparations and Corsodyl® proved to be of higher antifungal efficacy than Curasept® and Paroex®.

Conclusion:

Within the limitations of the study, it can be concluded that both pure and alcohol containing CHX preparations (Corsodyl®) are more potent against *C.albicans* than alcohol-free CHX preparations (Curasept® and Paroex®). However, in mixed cultures (facultative and strict anaerobes), alcohol-free CHX preparations (Curasept® and Paroex®) have antimicrobial efficacies comparable to that of both alcohol-containing (Corsodyl®) and pure CHX formulations (0.2% and 0.12%).

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DECLARATION

I hereby declare that "The antimicrobial efficacy of three chlorhexidine mouth rinses: an *in-vitro* analysis" is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

BasheerAbdalrahman

May 2014

Signed:……

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DEDICATION

I dedicate this work to my parents, for their unconditional support and boundless love.

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LIST OF ABBREVIATIONS

ADS	Anti-discolouration system
ANOVA	Analysis of Variance
ANO2	Anaerobic
BHI	Brain Heart Infusion
C	Carbon
C.albicans	*Candida albicans*
CFU	Colony forming units
Cl	Chlorine
CHX	Chlorhexidine
Cm	Centimetre
CPC	Cetylpyridinium chloride
E.Coli	*Escherichia coli*
FCLT	Facultatively anaerobic
FDA	Food and Drug Administration
H	Hydrogen
Hyd	Hydrogenated
S.mutans	*Streptococcus mutans*
Ph	Power of Hydrogen
p. value	Probability
Ml	Millilitre
Mg	Milligram
MIC	Minimum Inhibitory Concentration
Mm	Millimetre
N	Nitrogen
NaF	Sodium Fluoride
NCTC	National Collection of Type Cultures
Nm	Nanometer
PEG	Poly Ethylene Glycol
Sig.	Significance
Std	Standard deviation

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S.mutans	*Streptococcus mutans*
S.salivarious	*Sterptococcus salivarious*
UWC	University of the Western Cape
µl	Microliter

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CHAPTER 1

INTRODUCTION

Chlorhexidine (CHX) has been the gold standard for evaluating new chemical plaque control agents (Jones, 1997). In this study, three locally available CHX products in South Africa were tested *in vitro* against two preparations of pure CHX, to compare their antimicrobial effects on:

1) Facultatively anaerobic cultures prepared from oral rinse samples.

2) Strictly anaerobic cultures prepared from the same oral rinse samples.

3) Pure cultures of *S. mutans* prepared from the laboratory.

4) Pure cultures of *C. Albicans* prepared from the laboratory.

Commercially available CHX formulations differ in their concentrations as well as in the component additives present. Most CHX oral rinses are prepared in two concentrations: 0.2% and 0.12%. Some CHX preparations have alcohol added in concentrations as high as 14-15%, while others are alcohol free, the addition of which is controversial because of its carcinogenic potential and tissue irritating properties (Herrera *et al.,* 2003).

Some CHX preparations contain additional antimicrobials, like Cetylpyridinium chloride (Sreenivasan *et al*., 2012). Others have chemicals added to prevent teeth discolouration; a common side effect with prolonged use of CHX. It has been suggested that alterations to CHX formulations may have an effect on its antimicrobial efficacy (Herrera *et al*., 2003).

Previous studies examining different CHX preparations have shown a lack of consensus regarding the effect of additives on the antimicrobial efficacies of the different preparations (Guggenheim and Meier, 2011, Arweiler *et al*., 2006 & Herrera *et al*., 2003). This makes it imperative to test any CHX formulation that contains additives against well-studied and documented CHX formula.

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CHAPTER 2

LITERATURE REVIEW

2.1 Chlorhexidine:

Dental plaque is the primary aetiological factor implicated in dental caries and periodontal disease. It is a biofilm of different bacterial species protected by an extracellular polysaccharide matrix, which enables it to adhere to tooth and soft tissue surfaces. Early in plaque formation, the prominent organisms are Gram positive aerobic bacteria, collectively referred to as the primary colonizers. As the biofilm matures, more Gram negative facultative anaerobes and obligatory anaerobes, including spirochetes, accumulate. These bacteria are secondary colonizers. This matures further and becomes a complex ecosystem and affords biological advantages to all the organisms involved. It facilitates the exchange of nutrients and waste products. It also resists the diffusion of antimicrobials and other harmful, potent microbicidals into the biofilm (Marsh *et al*., 2011).

The first scientifically proven study demonstrating the effect of chlorhexidine on dental plaque microbes was performed in 1970 by Loë and Schiott. They showed that rinsing the mouth with 10 ml of 0.2% chlorhexidine for 60 seconds twice a day for 10 days, in the absence of mechanical plaque control, prevented the build-up of dental plaque and the subsequent development of gingivitis (Loë and Schiott, 1970).

The antibacterial effect of CHX has a wide spectrum. It is effective against both Gram positive and Gram negative organisms, but with different susceptibilities (expressed as different minimum inhibitory concentrations - MIC). Species with low MIC include *Staphylococci*, *S. mutans*, *S. salivarius* and *E. coli*, while the most resistant strains are Gram-negative *cocci* such as *Veillonella* (Emilson, 1977).

CHX is active not only against bacterial plaque species, but also against fungi (Gomes *et al*., 2013). *Candida* species form part of the normal microbial flora of the oral cavity in 15-75% of healthy individuals (Ten Cate *et al*., 2009). The ratio increases to 80% in geriatric patients wearing dentures (Vanden *et al*., 2008). *C. albicans* is by far the most common fungal species encountered in mycotic infections (Kraneveld *et al*., 2012).

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2.1.1 Chemical Structure:

By the end of the 1940s, British scientists were involved in developing potent antimalarial drugs. An English company, The Imperial Chemical Industries Limited, managed to synthesize a group of compounds known collectively as the polybiguanides that demonstrated a broad spectrum of antimicrobial activity (Lindhe *et al*., 2008). Further explorations of the chemical structure of the polybiguanides led in the 1950s to the synthesis of the bisbiguanides, which surpassed the original compounds by virtue of its wide antimicrobial spectrum. From that group of compounds, and through modifications to the chemical formulae at hand, it was possible to synthesize the compound with the highest bacteriostatic and bactericidal effects. That compound (1, 6, bis-4, chloro, phenyldiguanidohexane), became known as chlorhexidine, a very strong cationic compound (Davies *et al*., 1954).

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Figure-1: Chlorhexidine molecule: two symmetrical 4-chlorophenyl groups and two bisguanides groups linked by a hexamethylene bridge between them (Davies *et al*., 1954).

2.1.2 Uses in Dentistry:

When used prophylactically as an adjunct to mechanical debridement, CHX can reinforce the effects of mechanical plaque control by preventing adhesion and accumulation of dental plaque onto clean surfaces. It was found that warm mouthwashes were more efficacious than cold ones at reducing the oral microbial load (König *et al*., 2002). Due to the high susceptibility of *S. mutans* to CHX, it is commonly used to control dental caries in patients who prove to be in the high risk group (Gupta *et al*., 2012).

Dental prosthetics and orthodontic appliances can retain dental plaque and hamper mechanical plaque control. Thus disinfecting complete or partial dentures by immersing them in 0.2 %chlorhexidine solution at night can decrease the incidence of denture stomatitis (Gupta *et al*., 2012).

Recently, the postoperative use of CHX mouth rinses has replaced periodontal packs as the standard periodontal surgical care used to enhance healing in an infection-free environment. Other periodontal applications of CHX include its adjunctive use in total mouth disinfection, and as a substitute for saline in cooling ultrasonic tips (Lindhe *et al.*, 2008).

Patients with inter-maxillary fixation and those who are mentally challenged will benefit from the antimicrobial effects of CHX as a substitute for and adjunct to mechanical plaque control (Gupta *et al*., 2012).

The incidence and duration of minor aphthous ulcers are reportedly decreased following CHX use. This effect can be related to CHX's ability to control superimposed secondary bacterial infections (Gupta *et al.*, 2012).

Other reported uses of CHX include using it as a root canal disinfectant, for the treatment of halitosis and as disinfectant prior to performing oral surgical procedures. Non-dental uses include its use in ocular infections (in form of eye drops) and as a bio-adhesive vaginal gel in the treatment of bacterial vaginitis (Gupta *et al*., 2012).

2.1.3 Mechanism of Action:

Bacterial cell membranes contain phosphate groups that render them negatively charged. The strong positive charge of the CHX molecule is electrostatically attracted to negatively charged bacterial surfaces. This interaction damages the physical integrity of the bacterial cell membrane and results in leakage of cytoplasmic solutes, such as potassium. This effect is usually observed at low concentrations of CHX, and it is responsible for the bacteriostatic property of CHX. At higher concentrations, CHX precipitates cytoplasmic proteins and becomes microbicidal to organisms exposed to it (Mathur *et al.*, 2011& Gomes *et al.*, 2013). The actual concentrations at which the effect is bacteriostatic or bactericidal varies according to the bacterial species under investigations (Denton, 1991).

The adherence of the positively charged CHX to the negatively charged hard and soft tissue surfaces also explains its ability to withstand the flushing effect of saliva and other fluids in the oral cavity, long after its application. This phenomenon, termed substantivity, ensures the slow, continual release of CHX from oral tissue surfaces that makes its effects last for several hours (Gomes *et al.*, 2013).

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Since CHX is a base, its aqueous solution is chemically more stable at pH ranges between 5 and 8. Moreover, its antimicrobial activity is pH dependent. This activity is optimum between pH 5.5 and 7, which is similar to that of body fluids such as saliva (Gomes *et al*., 2013).

2.1.4 Toxicity and side effects:

Systemic absorption of CHX via the mucosa of the gastrointestinal tract is virtually non-existent. This is due to the hydrophobic nature of the cationic CHX molecule (Gupta *et al.,* 2012). As a result, all side effects of CHX are local reactions. These include the brownish staining of the teeth and dorsum of the tongue encountered after relatively short use (10-15 days) and taste disturbances, particularly salty taste (Lindhe *et al*., 2008). Epithelial desquamation can occur and in some patients, soft tissue lacerations have been reported after prolonged exposure. Parotid salivary gland swelling has only occasionally been reported. Lastly, CHX enhances supra-gingival calculus formation. This is attributed to its ability to precipitate salivary proteins, thereby accelerating pellicle formation. Used appropriately CHX is generally considered to be safe (Gupta *et al*., 2012).

2.1.5 *In-vitro* studies demonstrating decreased antimicrobial efficacy of different CHX preparations in the presence of non-alcoholic additives:

Several *In vitro* studies evaluated the antimicrobial effects of commercially available mouthrinses containing CHX. A Swiss study (Guggenheim and Meier, 2011) compared different CHX mouth washes with and without Anti Discolouration Systems (ADS) to Listerine® (**Table 1**). Water and pure CHX -in the form of an aqueous solution of 0.15% concentration- were used as negative and positive controls respectively. The results showed that the antimicrobial effects of Curasept® (non-alcohol CHX 0.12% with ADS), and Parodentosan® (CHX 0.05% with 15% alcohol) were 10,000 times weaker than PlakOut® rinse solution (CHX 0.1% with 8% alcohol), PlakOut® liquid (CHX 0.2% with 45% alcohol) and the positive control (pure CHX 0.15%). According to this study, both CHX formulations with chemical additives in the form of ADS i.e. the alcohol containing Parodentosan® and the alcohol free Curasept®, exhibited a lower antimicrobial efficacy than CHX formulations without ADS (PlakOut®) and pure CHX. It was concluded that "it is not possible to formulate CHX mouthwash preparations that have effective anti-discolouration systems without negatively affecting its antimicrobial efficacy" (Guggenheim and Meier, 2011).

Another study (Herrera *et al*., 2003) compared the antimicrobial effects of different CHX products with and without alcohol. Preparations used included PerioAid® (CHX 0.12% with 5% alcohol), PerioAid® Sin Alcohol® (non-alcohol CHX 0.12% with 0.05% CPC), Cariax Gingival® (non-alcohol CHX 0.12% with Sodium NaF) and Chlorhexidina Lacer® (non-alcohol CHX 0.12%) (**Table 1**). The study concluded that CHX preparations that contained alcohol showed enhanced antimicrobial activity *in vitro*, however the possibility of other additives imparting an antimicrobial effect could not be entirely excluded (Herrera *et al*., 2003).

2.2 Anti-Discolouration System:

ADS is a term used to encompass a number of chemical compounds used collectively to prevent the brown discolouration encountered with prolonged use of CHX (Bernardi *et al*., 2004). They are added to various products with different active ingredients. Prominent ADS compounds include ascorbic acid and sodium sulphite (Cortellini *et al*., 2008).

2.3 Cetylpyridinium Chloride:

Cetylpyridinium Chloride is a quaternary ammonium compound with a strong cationic nature that is readily attracted to negatively charged surfaces (Cortesia *et al*., 2010). CPC is typically active against Gram positive bacteria and fungi, with demonstrable bactericidal effects (Liu *et al*., 2013). The FDA classifies CPC amongst the safest and most effective chemical agents (class I for safety, and class I for efficacy) when used as a disinfectant in concentrations ranging from 0.045% - 0.10% (Sreenivasan *et al*., 2012). Most CPC formulations have 0.05% concentration. However, its efficacy was found to be affected by the presence of other chemicals that are commonly added to the formula as excipients such as ethanol, sorbitol, glycerine, propylene glycol and monosodium phosphate (Fathilah *et al*., 2012). A commercially available mouthwash, Paroex® contains 0.05 % CPC.

2.4 Alcohol:

Some authors are of the opinion that the addition of alcohol to CHX solutions is necessary for chemical stability, improved antimicrobial efficacy and to prevent its contamination (Vigeant *et al*., 1998). The argument against adding alcohol is threefold: 1) the well-known carcinogenic potential of ethanol (Elmore and Horwitz, 1995). In alcoholics, the use of alcohol containing mouthwashes increases the risk of developing oropharyngeal cancer (Winn *et al*., 1991). 2) the tissue irritating properties, which precludes its use in radiation or

chemotherapy damaged epithelial surfaces (Ennibi *et al*., 2013). In patients under chemotherapy or radiation therapy, symptoms of mucositis -if present- will be aggravated; and 3) Alcohol allergy (Eldridge *et al*., 1998). Alcohol is known as an allergen that can induce hypersensitivity reactions in susceptible individuals (Rajan and Khan, 2010).

2.5 Streptococcus mutans:

S. mutans is the most cariogenic intra-oral bacteria (Mai *et al.,* 2011). The Gram positive, facultatively anaerobic coccus is one of the primary colonizers in plaque formation (Lindhe *et al*., 2008). The organism adheres to the salivary proteins, epithelial cells and polymorphonuclear cells covering tooth surfaces –i.e. the dental pellicle- shortly after its formation. Dental plaque samples taken 24 hours after mechanical cleaning consists mostly of *Streptococci* (Lindhe *et al*., 2008).

2.6 Candida albicans:

More than 150 species are found in the *Candida* genus. Strains of *Candida* are part of the normal oral flora in healthy individuals. C*andida albicans* is an opportunistic pathogen that frequently affects medically compromised individuals, and denture wearers (Pereira-Cencip *et al*., 2008).The fungus is dimorphic, i.e. it can exist in either a yeast or hyphal form, depending on the environmental conditions (Farah *et al*., 2010). The clinical variants of oral candidiasis includes: acute pseudomembranous, chronic atrophic, chronic erythematous (including angular cheilitis and denture stomatitis) or chronic hyperplastic candidiasis (Williams and Lewis, 2011).

2.7 Facultative anaerobes:

These organisms can grow in the presence or absence of oxygen and develop during the early phases of dental plaque formation. Beside *Streptococci, Actinomyces* species are among the major primary colonizers that consume oxygen, thereby paving the way for strictly anaerobic secondary colonizers (Marsh *et al*., 2011). These Gram positive rods are initially found in low numbers, gradually increasing to eventually outnumber *streptococci* (Lindhe *et al*., 2008).

2.8 Strict anaerobes:

They cannot grow in the presence of oxygen and are secondary colonizers in dental plaque. They are mostly Gram negative rods and are strict anaerobes, such as *Porphyromonas gingivalis* and *Tannerella forsythia* (Marsh *et al*., 2011). These organisms possess an array of proteolytic enzymes and other virulence factors that define them as the major periopathogens (Lindhe *et al*., 2008)

Table 1: CHX products tested in previous studies.

Study	Product & Manufacturer	Active Ingredients	Additives
Guggenheim & Meier, 2011	PlakOut®, (KerrHawe SA, Bioggio)	CHX digluconate 0.1%	Ethanol 8% v/v, flavouring, dye: E127
	PlakOut®, (KerrHawe SA, Bioggio)	CHX digluconate 0.2%	Ethanol 45 vol.%, flavouring
	Curasept® (Curaden Health-Care, Saronno (VA), Italy)	CHX digluconate 0.12%	Xylitol, propylene glycol, PEG 40, hyd. castor oil, ascorbic acid, Poloxamer 407, sodium metabisulfite sodium citrate, aroma Cl.42090
	Curasept® (Curaden Health-Care, Saronno (VA), Italy)	CHX digluconate 0.2%	Xylitol, propylene glycol, PEG 40, hyd. castor oil, ascorbic acid, Poloxamer 407, sodium metabisulfite sodium citrate, aroma Cl.42090
	Parodentosan® (Tentan AG, Ramlinsburg)	CHX digluconate 0.05%	Per ml: Ethanol 15 vol.%, myrrh tincture 1.9 mg, sage oil 0.5 mg, peppermint oil 0.08 mg, xylitol and other adjuvants
	Listerine® (Johnson & Johnson, Maidenhead UK)	Thymol 0.064%, Menthol 0.042% Eucalyptol 0.060%	Ethanol 21%, Sorbitol, 1-propanol, methylsalicylate, Poloxamer 407, benzoic acid Cl l47005, sodium fluoride 100 ppm, and others
	Pure CHX (Sigma-Aldrich, ChemieGmbh, D-Steinheim 88552) – Positive Control	CHX digluconate 0.15%	
	Water – Negative Control		
Herrera *et al*., 2003	PerioAid® (Dentaid, Spain)	CHX 0.12%	Ethanol 5%
	PerioAid Sin Alcohol® (Dentaid, Spain)	CXH 0.12%	0.05% Cetylpyridinium Chloride
	Cariax Gingival® (Kin SA, Spain)	CHX 0.12%	0.05% Cetylpyridinium Chloride
	Chlorhexidina Lacer® (Lacer SA, Spain)	CHX 0.12%	

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CHAPTER 3

Aim and Objectives

3.1 Aim:

To compare the antimicrobial efficacy of three locally available CHX mouth rinses.

3.2 Objectives:

To measure the antimicrobial efficacy of:

1. Curasept®: an alcohol free, 0.2% CHX gluconate, with ADS in the form of chemical additives.

2- Paroex®: an alcohol free, 0.12% CHX gluconate with 0.05% CPC.

3- Corsodyl®: 0.2% CHX gluconate with 5% alcohol.

Against 1) *Streptococcus mutans*, 2) *Candida* albicans, *3*) Facultative anaerobes and 4) Strict anaerobes

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CHAPTER 4

METHODOLOGY

4.1 Study design:

This study was an *in vitro* analytical study of an exploratory nature.

4.2 Study sample:

All staff members at the University of the Western Cape, Dental Faculty were invited to participate in the study. Every alternate staff member who presented between 2:00pm and 4:00 pm on 2 consecutive Mondays, were selected to participate. Oral rinse samples were collected from 14 staff members, who met the inclusion criteria.

4.2.1 Inclusion criteria:

1- Dentate and partially dentate individuals.

2- Persons above 12 years of age.

3- Systemically healthy.

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4.2.2 Exclusion criteria:

1- Edentulous individuals.

2- Children below 12 years old.

3- Patients with systemic conditions.

4- Smokers.

5- Individuals who have used antibiotics or immunosuppressive drug therapies during the past 3 months.

6- Persons with active periodontal disease.

7- Persons with active carious lesions.

8- Persons with oral candidiasis.

4.3 Materials

4.3.1 The CHX preparations:

The 3 commercially available mouthrinses were purchased from local stores, while the controls - (pure CHX preparations) - were obtained from the Institute of Oral and Dental Research at the Faculty of Dentistry, University of the Western Cape. These particular CHX formulations were chosen because –to the best of the author's knowledge- they were the most commonly found in local markets in Cape Town, South Africa.

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Figure 2: The 3 commercial CHX products; Corsodyl® (CHX3), Curasept® (CHX4) and Paroex® (CHX5)

The pure CHX formulation was in the form of an aqueous (water based) solution of 20% CHX concentration. Two different concentrations (0.2% & 0.12%) of alcohol-free pure CHX were prepared by titration with sterile water. The pure CHX 0.2% acted as a control for Corsodyl® and Curasept® (both having CHX 0.2% concentration), while pure CHX 0.12% was the control for Paroex® (CHX 0.12%).

Table 2: CHX products tested in the current study. *CHX1-CHX5: codes as used in the disk diffusion test*	
CHX preparation	**Active ingredients**
20% aqueous solution CHX digluconate (Sigma-Aldrich) – CHX1	CHX 0.2%
20% aqueous solution CHX digluconate (Sigma-Aldrich) – CHX2	CHX 0.12%
Corsodyl® - CHX3	CHX 0.2%, Ethanol 5%
Curasept® - CHX4	CHX 0.2%, ADS (Xylitol, propylene glycol, PEG 40, hyd. castor oil, ascorbic acid, Poloxamer 407, sodium metabisulfite sodium citrate, aroma Cl.42090)
Paroex® - CHX5	CHX 0.12%, Cetylpyridinium Chloride 0.05%

4.3.2 Digital calliper

A commercially available digital calliper was purchased. Readings were adjusted to two decimal places, and measuring units were centimetres.

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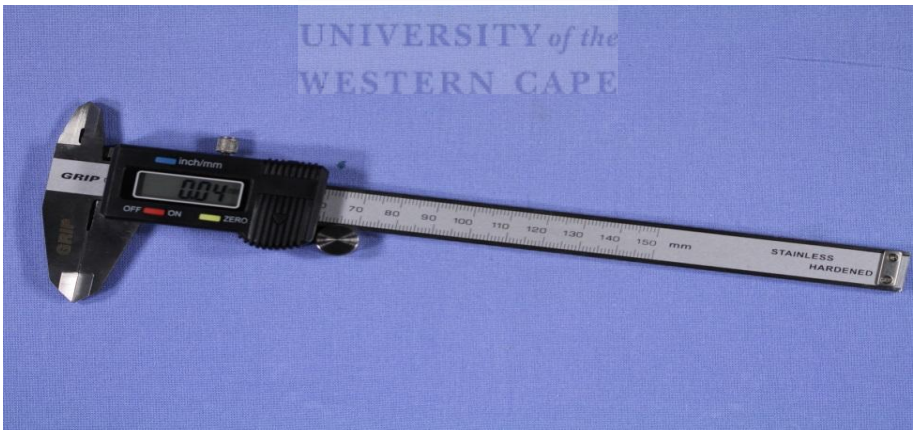

Figure 3: Digital calliper used to measure dimensions of inhibition zones

4.4 Ethical approval

Ethical approval was obtained from the UWC Dental Faculty. Individual participant consent for specimen collection was via informed consent. The voluntary nature of the participation in this study was clearly explained to the participants, along with any potential advantage, disadvantage, compensation or complaints that might result due to taking part in this study. The researcher's contact details were available to all participants for further information about the study or its outcome.

4.5 Conflict of Interest

This study was funded by the Faculty of Dentistry, University of the Western Cape. Neither financial nor any other kind of material support was offered by the manufacturers of the different products under investigation. The researcher thereby declares no conflict of interest in this study. Any relevant findings will be submitted for publications.

4.6 Specimen preparation and data collection

4.6.1 Collection and preparation of oral rinse samples

Oral rinse samples were collected from 14 staff members at the faculty of Dentistry, UWC, who met the inclusion criteria. Each subject was supplied with 10 ml of sterile saline in a universal container and instructed to rinse the mouth meticulously in the presence of the researcher for 60 seconds and then return the mouth rinse to the container (Samaranayake *et al*., 1986).

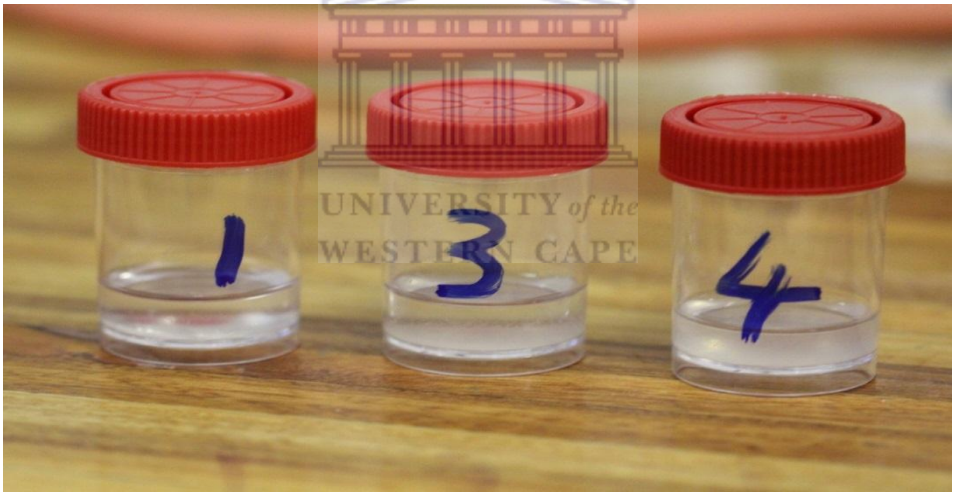

Figure 4: Universal containers each with 10ml of oral rinse solution collected from study participants – numbers on containers correspond to code numbers given to participants.

100µl of the rinse was inoculated onto previously prepared Brain Heart Infusion agar plates, 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* (Dzink *et al*., 1985). The anaerobic conditions were created inside an anaerobic jar utilizing an anaerobic system (Oxoid® Gas generating kit – made in UK), with Palladium as a catalyst. A colour indicator was used to signal the

transformation to anaerobic conditions. For the facultative anaerobic cultures, an anaerobic incubator was used. The incubation period for both types of cultures was 24 hours.

4.6.2 Preparation of pure cultures

Pure cultures of *S. mutans* and *C. albicans* were selected, as these microorganisms are known etiological factors for dental caries and *candidiasis* respectively. To this end, *S. mutans* NCTC 25175 and *C. albicans* NCTC 36801 type strains were cultured in the laboratory overnight (24 hours). Thereafter, a separate inoculum from each culture was prepared. This was done by selecting appropriate cultures and preparing a suspension thereof in saline using the direct colony suspension method.

The two suspensions (*S. mutans* & *C. albicans*) were standardized to 0.5 McFarland standard (corresponding approximately to 1.5 X 10^8 CFU/ml). The McFarland scale is used for measuring bacterial densities in suspensions (**Table 3**). 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.

Table 3: McFarland standards and corresponding cell counts.

McFarland Standard	Approximate Cell Count Density ($x10^8$cells)
0.5	1.5
1.0	3.0
2.0	6.0
3.0	9.0
4.0	12.0

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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 on the surface of the plate. This ensured a more or less balanced distribution of the bacteria in question throughout the surface of the 28 agar plates.

4.6.3 Disk Diffusion Test to measure inhibition zones

The 56 agar plates used for the disk diffusion test were divided equally into 4 groups as listed below:

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

The disk diffusion test was performed by adding 5 sterile, 6mm diameter, filter paper disks to each of the 56 BHI plates. Each disk was saturated with 10µl of the specified CHX products tested and assigned a code number corresponding to the CHX product used (**Figure 5**). The disks were evenly distributed on the agar surface. The antibacterial effects of each CHX product was measured in terms of the dimensions of the bacterial growth inhibition zone around the disks that occurred within 24hours of incubation (**Figure 6**).

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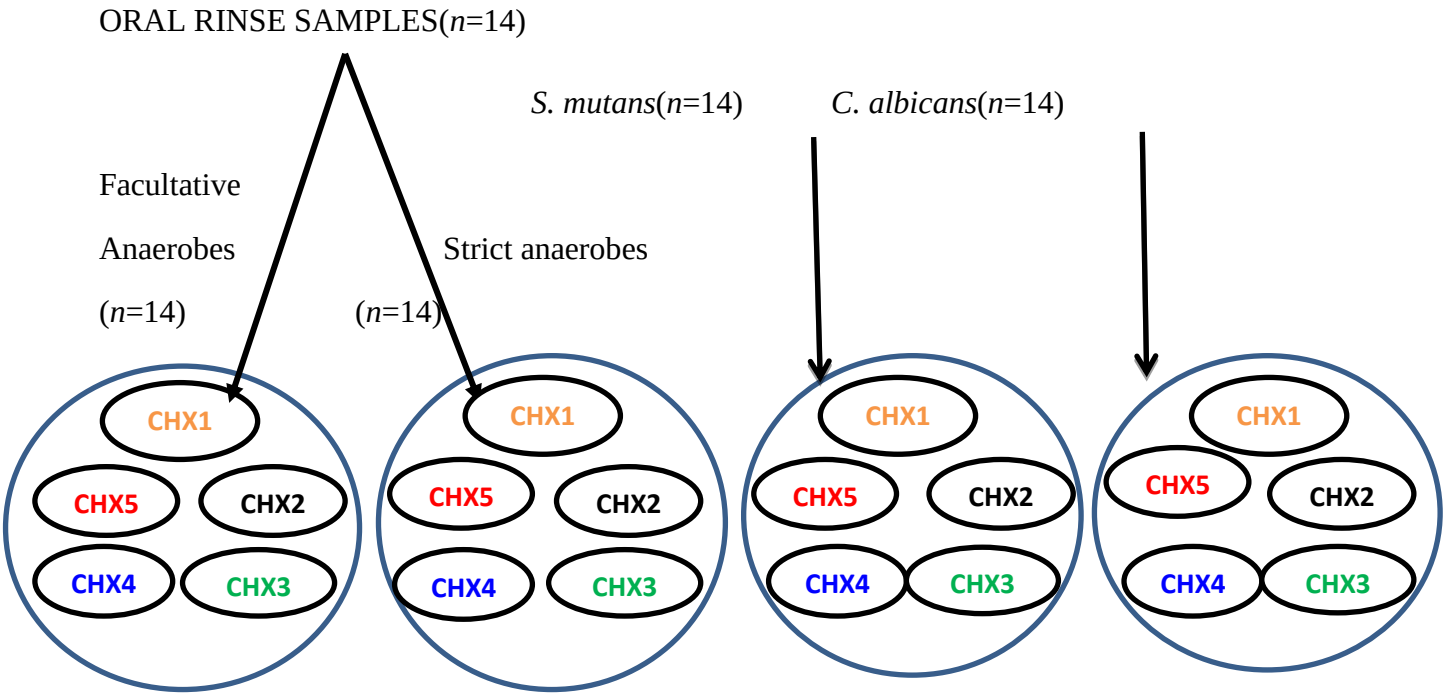

Figure 5: Agar plates and filter paper disks used in disk diffusion test illustrating the inhibition zone. *S. mutans* and *C.albican* were inoculated from pure cultures.

All measurements were executed by the principal investigator and a second clinician using a digital calliper (**Figure 3**). The diameter of each inhibition zone was measured three times, by each investigator, who was blinded to the preceding measurement. The readings were averaged and those with a discrepancy of more than 1mm were discarded and re-measured. Data capturing tables (**Appendix 3**), were used to record the readings, from which the mean and standard deviation were calculated.

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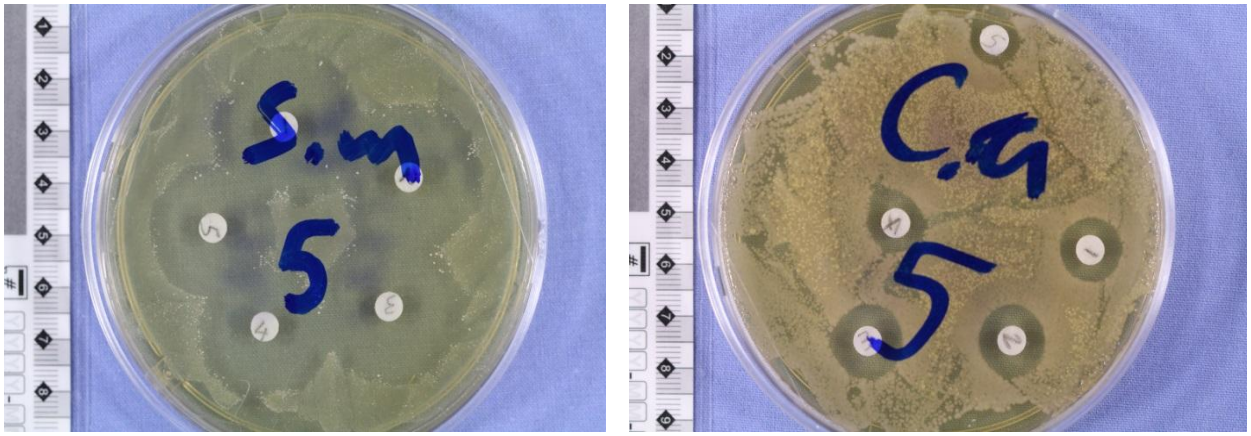

Figure 6: BHI agar plates no.5 - (out of 14 for each) - showing *S.mutans* and *C.albicans* pure cultures with inhibition zones around different CHX preparations. Numbers on filter papers denotes the following: - 1: CHX1 (Pure CHX 0.2%), 2: CHX2 (Pure CHX 0.12%), 3: CHX3 (Corsodyl®), 4: CHX4 (Curasept®) and 5: CHX5 (Paroex®)

4.7 Data Analysis:

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

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CHAPTER 5

RESULTS

5.1 Demographic characteristics of participants:

All of the 14 volunteers participating in the study were staff members of UWC Dental Faculty. The majority of the participants were females (71.43%), with males only making up 28.57% of the sample. The age range for the study participants was 19-68 years, with a mean age of 38 years (standard deviation =16.84).

5.2 Means of inhibition zones' diameters:

The mean inhibition zone for each CHX disk per agar plate was calculated from the 3 measurements recorded. A second mean (group mean) was calculated from the average of all 14 disks impregnated with the same CHX preparation.

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Table 4: Group means and Standard Deviations for inhibition zones (in cm)

n=14	pure CHX 0.2%	pure CHX 0.12%	Corsodyl®	Curasept®	Paroex®
S.mutans	2.48± 0.37	2.31± 0.47	2.39± 0.44	2.15± 0.39	2.10± 0.32
C.albicans	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

Corsodyl® and Curasept® both comprise a 0.2% CHX concentration with additives; they were compared to a pure CHX formulation of the same concentration. **Figure 7** compares their inhibition zones for the 4 culture types.

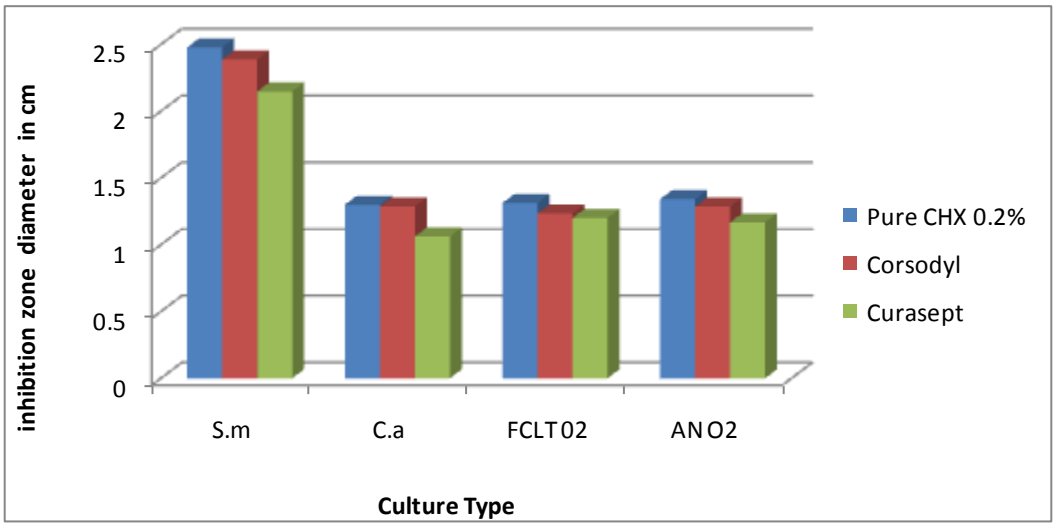

Figure 7: Different means of inhibition zones attained by the 3 different CHX 0.2% preparations for the 4 culture types.

Curasept® produced the lowest readings in all types of culture, while Corsodyl® scored better readings than Curasept®, but still lower than the pure CHX 0.2% formulation.

When Paroex® (CHX 0.12% + CPC 0.05%) was compared to pure CHX 0.12%, the following results were found **(Figure 8)**.

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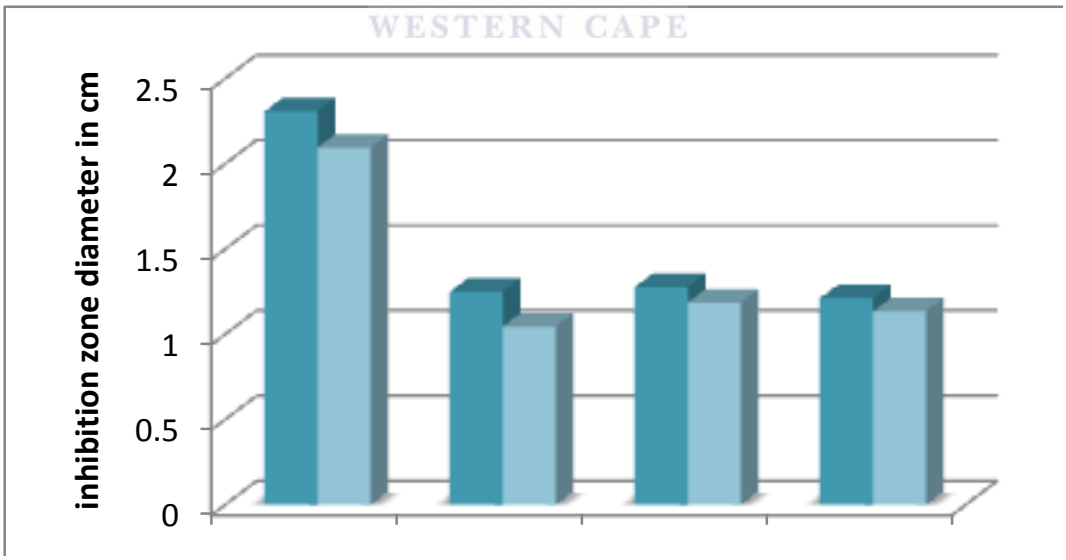

Figure 8: Different means of inhibition zones attained by the 2 different CHX 0.12% preparations.

When the means of inhibition zones for all CHX formulations were considered together, readings could thus be represented as in **Figure 9**.

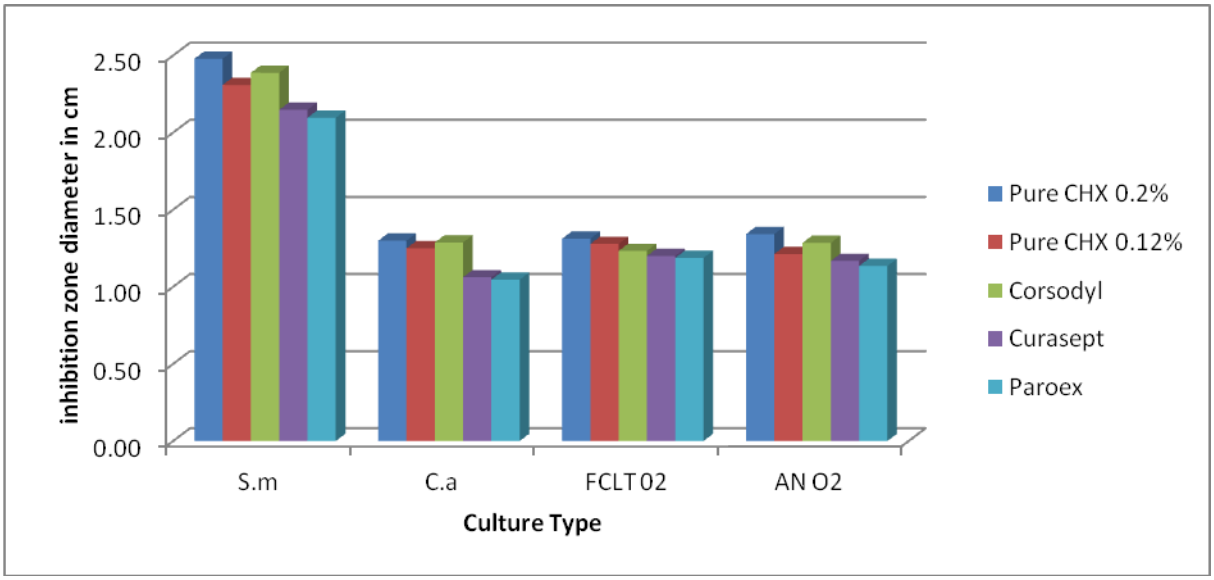

Figure 9: Different means of inhibition zones attained by all CHX preparations.

Figure 10 represents the performance of each CHX formulation across the 4 different types of cultures. *Streptococcus mutans* cultures were clearly more sensitive to all CHX formulations than other cultures, while *Candida albicans* cultures showed sensitivity comparable to that of facultative and strict anaerobic cultures gotten from study participants.

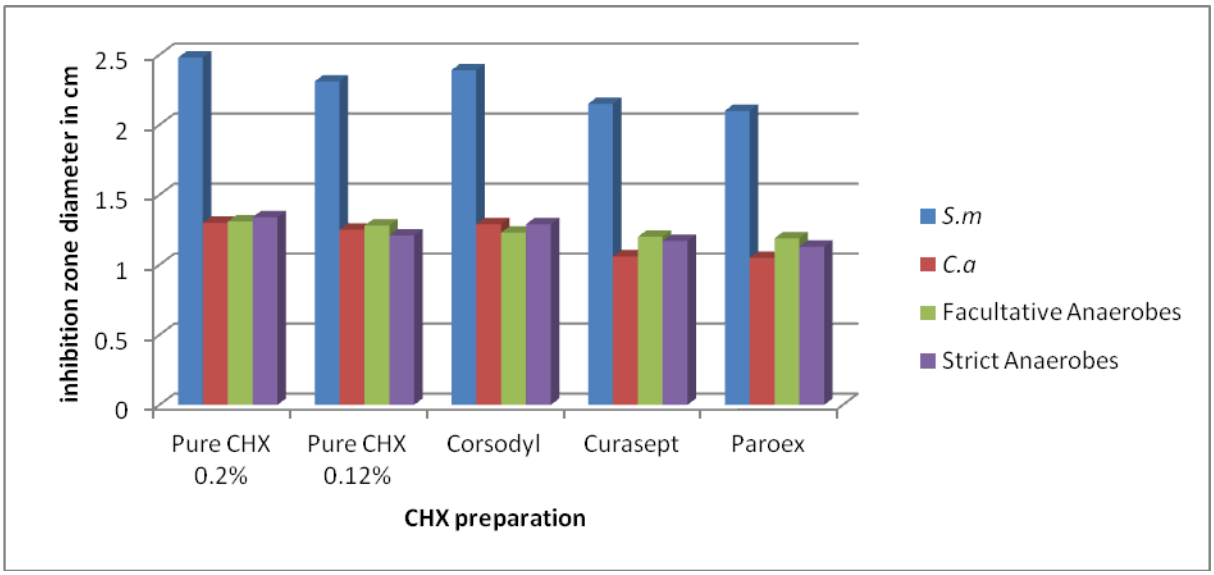

Figure 10: different means of inhibition zones for all 4 types of cultures as occurred with each CHX preparation.

5.3 ANOVA tests results:

To verify whether these differences were statistically significant or not, ANOVA one way statistical test was calculated for each of the 4 cultures, whereby it was found that the only statistically significant difference (*p*. value <0.05) between the means of the diameters of inhibition zones attained by the different CHX formulations was present in *Candida albicans* cultures as shown in **Table 5**.

Table 5: ANOVA one way test results for each type of culture

Culture type	ANOVA result	Statistically significant
Streptococcus mutans	0.073	no
Candida albicans	0.0001	yes
Facultative anaerobes	0.867	no
Strict anaerobes	0.391	no

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5.4 *Post-hoc* analysis: Tukey and Bonferroni tests (*C.albicans*)

The only statistically significant difference between all the means of inhibition zones was found in the group of *C.albicans* cultures. Multiple comparisons tests (Tukey and Bonferroni) were chosen for post-hoc analysis to measure statistical significance within the group (**Tables 6 & 7**).

Both tests (Tukey and Bonferroni) showed that the 5 CHX formulations could be categorized into 2 groups based on the presence or absence of a statistically significant difference between the means of inhibition zones for all possible pair combinations. The differences between the 2 pure CHX preparations and Corsodyl® were not statistically significant, thereby all 3 CHX formulations (pure CHX 0.2%, pure CHX 0.12% and Corsodyl®) were considered to be of a comparable antimicrobial efficacy and constituted one group. The comparison between Curasept® and Paroex® showed no statistical significance in their antimicrobial efficacy against *C.albicans*, hence both alcohol-free CHX formulations constituted a separate group. However, each and every group member showed a statistically significant difference when compared to any member of the other group. Accordingly, Corsodyl® showed a statistically significant difference (i.e. higher means of diameters of inhibition zones) from both Curasept® and Paroex®, which means that its antimicrobial efficacy against *C.albicans* is higher than both preparations.

Table 6: Multiple comparisons within *C.albicans* group by Tukey test		
Type	**Type**	**Sig.**
1.00 CHX1	CHX2	0.253
	CHX3	0.970
	CHX4	0.000
	CHX5	0.000
2.00 CHX2	CHX1	0.253
	CHX3	0.614
	CHX4	0.000
	CHX5	0.000
3.00 CHX3	CHX1	0.970
	CHX2	0.614
	CHX4	0.000
	CHX5	0.000
4.00 CHX4	CHX1	0.000
	CHX2	0.000
	CHX3	0.000
	CHX5	0.970
5.00 CHX5	CHX1	0.000
	CHX2	0.000
	CHX3	0.000
	CHX4	0.970

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The result is statistically significant when sig. = 0, highlighted in yellow.

CHX1: Pure CHX 0.2%

CHX2: Pure CHX 0.12%

CHX3: Corsodyl® (0.2% CHX + 5% Alcohol)

CHX4: Curasept® (0.2% CHX + ADS)

CHX5: Paroex® (0.12% CHX + 0.05% CPC)

Table 7: Multiple Comparisons within *C.albicans* group by Bonferroni test		
Type	**Type**	**Sig.**
1.00 CHX1	CHX2	1
	CHX3	1
	CHX4	0
	CHX5	0
2.00 CHX2	CHX1	1
	CHX3	1
	CHX4	0
	CHX5	0
3.00 CHX3	CHX1	1
	CHX2	1
	CHX4	0
	CHX5	0
4.00 CHX4	CHX1	0
	CHX2	0
	CHX3	0
	CHX5	1
5.00 CHX5	CHX1	0
	CHX2	0
	CHX3	0
	CHX4	1

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The result is statistically significant when sig. = 0, highlighted in yellow.

CHX1: Pure CHX 0.2%

CHX2: Pure CHX 0.12%

CHX3: Corsodyl® (0.2% CHX + 5% Alcohol)

CHX4: Curasept® (0.2% CHX + ADS)

CHX5: Paroex® (0.12% CHX + 0.05% CPC)

CHAPTER 6

DISCUSSION

6.1 Introduction:

In this study, pure cultures prepared from strains available in the laboratory(*S. mutans* NCTC 25175 and *C. albicans* NCTC 36801 type strains), and mixed cultures (facultative and strict anaerobes) prepared from oral rinse samples obtained from 14 study participants, were utilized to compare the antimicrobial efficacy of 3 different CHX preparations.

Regarding oral microbial flora specimens taken from study participants, oral rinse samples were cultured in such a way that made it possible to differentiate between facultative anaerobes and strict anaerobes. These, collectively form dental plaque, the primary aetiological agent behind dental caries and periodontal disease. Previous studies have shown oral rinse samples to contain a representative sample of all oral microorganisms present, including periodontal pathogens found in periodontal pockets (Samaranayake *et al*., 1986).

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The 3 different CHX formulations under investigation were Corsodyl®, Curasept® and Paroex®. Since the 3 products have different CHX concentrations, a positive control (pure CHX), was similarly prepared in 2 different concentrations (0.2% and 0.12%).

6.2 Streptococcus mutans cultures*:*

Results indicate that antimicrobial efficacies of all CHX formulations were highest against *Streptococcus mutans*, when compared to other cultures (**Figures 9 & 10**). This reflects the fact that CHX has a potent anti-cariogenic effect that can allow its use as an adjuvant to mechanical oral hygiene measures (Emilson, 1977).

The order of antimicrobial efficacy found for the different CHX formulations in descending order was:

1- Pure CHX 0.2%
2- Corsodyl® (CHX 0.2% + Alcohol 5%)
3- Pure CHX 0.12%

4- Curasept® (CHX 0.2% + ADS)
5- Paroex® (CHX 0.12% + CPC 0.05%)

Corsodyl®, containing 5% alcohol, exhibited a lower means (i.e. lower antimicrobial efficacy) than pure CHX 0.2%, but higher than Curasept® which has a similar CHX concentration, but is alcohol free.

The finding that Curasept®, which is alcohol free, scored lower means than pure CHX 0.2%, as well as pure CHX 0.12%, supports the previous findings that additives (such as ADS) could decrease the antimicrobial efficacy of CHX (Guggenheim and Meier, 2011). This could be due to the strong positive charge of CHX molecule that renders it highly reactive with negatively charged molecules, whether they were chemical additives or biological molecules in cell membranes (Gomes *et al*., 2013).

Even though Paroex® contains 0.05% CPC in its formula, it was surpassed in antimicrobial efficacy by pure CHX 0.12%. This can also be attributed to the aforementioned chemical interactions between CHX and any additives. However, the addition of 0.05% CPC to the formula of Paroex® (0.12% CHX) was possibly the cause of its antimicrobial efficacy being comparable to Curasept (0.2% CHX).

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Nevertheless, these results are not conclusive, and should be dealt with cautiously since the results of ANOVA test comparing the means of inhibition zones attained by different CHX formulations was not statistically significant (*p*. value = 0.073). Increasing the sample size for *S.mutans* cultures could have decreased the margin of error.

6.3 C. albicans cultures:

The means of the inhibition zones' diameters in all 14 *C. albicans* cultures were lower than recorded for *S. mutans* cultures, indicating lower susceptibility of *C.albicans* to all CHX formulations under investigation. This is a consistent result when taking into consideration the greater complexity of the fungal cell membranes compared to that of Gram positive bacteria (Chaffin, 2008).

The order of antimicrobial efficacy for the different CHX formulations from highest to lowest was:

1- Pure CHX 0.2%
2- Corsodyl® (CHX 0.2% + Alcohol 5%)

3- Pure CHX 0.12%
4- Curasept® (CHX 0.2% + ADS)
5- Paroex® (CHX 0.12% + CPC 0.05%)

Although this order was similar to that observed in the *S. mutans* cultures, the differences were statistically significant (*p*. value = 0.0001). Consequently, the interpretation of these differences have more credibility than for *S. mutans* cultures.

Results clearly indicate that Curasept® and Paroex® are less active than Pure CHX and Corsodyl® against *C. albicans*. This supports a synergistic antimicrobial role for preparations containing both alcohol and CHX against *Candida*. This is further supported by the results of the means (**Table 4**). Even though in all the different types of cultures, pure CHX 0.2% always scored a higher means than Corsodyl®, the difference was only marginal for *Candida albicans* cultures (1.30cm for pure CHX 0.2%, 1.29cm for Corsodyl®).

For both *S.mutans* and *C.albicans*, the readings across the 14 cultures were numerically closer than in both types of cultures prepared from participants' oral rinses. Such variability reflects the qualitative differences in oral microbial flora from an individual to another.

6.4 Facultative anaerobic cultures:

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Based on the result of ANOVA test, differences between the antimicrobial efficacies of all CHX formulations were found to be statistically insignificant (*p*. value of 0.867). Therefore, all tested CHX formulations have comparable antimicrobial efficacy against facultative anaerobic bacteria. According to this finding, it is plausible to assume that all tested CHX products might exhibit comparable antimicrobial efficacy *in-vivo*, where the dental plaque more or less matches -qualitatively- this type of culture.

6.5 Strictly anaerobic cultures:

No statistically significant difference could be elucidated by comparing the means of inhibition zones around the different CHX disks in each of the 14 anaerobic cultures.

In both groups of oral rinse cultures (facultative and strict anaerobes), obvious differences were noted in readings within groups, reflecting the different composition of flora between humans. Some individuals might have been harbouring organisms resistant to CHX more than others and in rare instances show complete resistance to a particular CHX product. The latter case was observed in sample 14 of the strictly anaerobic cultures where disks

impregnated with Paroex® were totally devoid of inhibition zones around them. A similar picture is seen with antibiotic resistance and maybe attributed to the development of resistance to CHX in this study.

The antimicrobial efficacies of Curasept® and Paroex® were better in both types of mixed cultures (i.e. both facultative and strict anaerobic) compared to their scores in pure cultures. It was due to this tendency (i.e. to affect mixed cultures more than pure ones) that it was not possible to spot a statistically significant difference between the different types of CHX in oral rinse cultures.

The overall sensitivity of oral rinse cultures (facultative and strict anaerobes) was comparable to that of pure cultures of *C.albicans* (**Figure 5**). Evidently, bacterial colonies become more efficient in resisting the effects of antimicrobials with increased compositional complexity (Lindhe *et al*., 2008)

It is noteworthy to mention that the most commonly resistant strains to all tested CHX preparations (growing in distinct colonies inside inhibition zones) were mostly large Gram positive *cocci*. However, it was not possible to rule out whether these strains were oral bacteria or acquired contaminants.

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The group that contained pure CHX with its 2 different concentrations (0.2% and 0.12%) and Corsodyl®, scored higher overall readings compared to the group of alcohol-free CHX formulations (Curasept® and Paroex®). This was a consistent pattern across all 4 types of cultures. Within the first group, Corsodyl® (0.2% CHX) showed higher means than pure CHX 0.12% in 3 out of 4 types of cultures (75% of time), while the means for Curasept® marginally higher than Paroex® in all 4 types of cultures.

Herrera *et al* concluded that CHX formulations that contained alcohol fared better than alcohol free CHX formulations (Herrera *et al*., 2003). In this study, that finding was validated with regard to *C. albicans* cultures. Another *in vitro* study (Guggenheim and Meier, 2011), employing a poly species biofilm, proved that pure or alcohol containing CHX formulations exhibit more potent antimicrobial properties than alcohol-free CHX preparations containing ADS as a chemical additive.

A common adverse effect of alcohol is its carcinogenic potential. These adverse effects are not expected to occur with concentrations below 25%, and are found to occur only when used on a daily basis for prolonged periods of time (Winn *et al*., 1991). The design of CHX

formulations that avoid the common side effects of the compound without affecting its antimicrobial efficacy remains to be a noble but elusive goal. For the while, patient risk benefit ratio should be evaluated when its use is indicated for a few days (Guggenheim and Meiers, 2011).

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CHAPTER 7

LIMITATIONS

There are some important limitations to the present study:

- Organisms suspended in oral rinse samples are in a planktonic state and do not exhibit the characteristics of the typical plaque biofilm. Dental plaque biofilms are expected to exhibit increased antimicrobial resistance (Lindhe *et al*., 2008). Previous studies used plaque biofilm models that needed complicated methods of preparations (Guggenheim and Meier, 2011 & Herrera *et al*., 2003).
- Mixed cultures (facultative and strict anaerobes) were dealt with collectively. The sensitivity of isolated oral bacterial species to CHX was not investigated since it was beyond the scope of this study.
- The study sample size was limited.
- Extrapolation of the current results to what is expected to occur in oral environments needs a complementary *in-vivo* clinical study.
- Even though a digital calliper was used to measure the diameters of inhibition zones, determining the boundaries of inhibition zones proved to be difficult and subjective at times.
- The presence of contaminant bacterial species could never be ruled out.
- The presence of non-*albicans* species of *Candida* (e.g. *C.tropicalis*, *C.parapsilosis*, *C.glabrata* and *C.krusei*) in oral rinse samples was not investigated. The role these species might play in conferring resistance of *C.albicans* to CHX should be investigated on a molecular level.

CHAPTER 8

CONCLUSIONS

- CHX formulations that contain alcohol (Corsodyl® in this study) are more potent against *C.albicans* than alcohol-free CHX formulations (Curasept® and Paroex®).
- Despite the difference in concentrations, Curasept® (0.2% CHX) and Paroex® (0.12% CHX) have similar antimicrobial efficacies. The addition of 0.05% CPC in Paroex®, or ADS in Curasept®, may support these results.
- Chemical Additives seem to negatively affect the antimicrobial efficacy of all CHX formulations, when compared to the corresponding concentration of pure CHX (only applicable in *C.albicans* cultures).
- In mixed cultures (facultative and strict anaerobes), alcohol-free CHX formulations (Curasept® and Paroex®) have antimicrobial efficacies comparable to that of both alcohol-containing (Corsodyl®) and pure CHX formulations (0.2% and 0.12%).
- Cultures prepared from human oral rinse samples demonstrated inter-individual variations in sensitivity to CHX formulations, i.e. the magnitude of antimicrobial activity of CHX differs from one oral flora to another, and is not universal.
- *S.mutans* cultures were sensitive to all CHX formulations more than other types of cultures (**Figure 6**), which translates into potent anticariogenic properties possessed by all formulations of CHX.
- *C.albicans* is more resistant/less sensitive to all CHX formulations than *S.mutans*.
- CHX formulations that contain alcohol should be reserved for *Candidal* infections. However, the minimum effective dose of alcohol in CHX formulations needs to be determined by further investigations.
- Since *S.mutans* is a primary colonizer, the anti-cariogenic effect of CHX is expected to be enhanced if applied immediately after tooth brushing.
- Curasept® (0.2% CHX) can be prescribed in a regimen similar to Paroex® (0.12% CHX), i.e. for prophylactic, long term use.

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- The pattern of inter-individual variations in the antimicrobial efficacy of CHX parallels the pattern observed in antibiotic sensitivity tests. This might herald the occurrence of resistant strains to CHX. Further studies are needed in this regard.
- Manufacturers should acknowledge the fact that any chemical additive can potentially decrease the antimicrobial efficacy of CHX.

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Appendix 1

Information Sheet

The antimicrobial efficacy of 3 chlorhexidine mouth rinses: an in-vitro analysis

I am Dr.BM Abdalrahman, a postgraduate dental student at the faculty of Dentistry, University of Western Cape.

I would like to invite you to take part in a research study. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Ask questions if anything you read is not clear or would like more information. Take time to decide whether or not to take part.

What is the purpose of the study?

This study is aiming to measure -in a laboratory setting- the antimicrobial effects of 3different chlorhexidine mouth washes. These mouthwashes are available in local markets and have been marketed as being effective in inhibiting oral bacteria to more or less similar degrees.

Why have I been invited?

You have been invited to participate in this research because you satisfy the inclusion criteria of the study, which states that individuals who are dentate (have the full set of teeth) or partially dentate, above 12 years of age and systemically healthy are eligible to participate. Sampling of participants is meant to be random, i.e. no specific ethnic group or gender is targeted more than the rest of the population. If you are a smoker, diabetic, pregnant, have other medical/genetic conditions (as will be explained by the examiner), under antibiotic treatment at the moment or during the past three months, or you have no natural teeth left, then you are unsuitable to participate in this study (but anyway, thanks for your time!).

Do I have to take part?

It is up to you to decide. We will describe the study and go through the information sheet, which we will give to you. We will then ask you to sign a consent form to show you agreed

to take part. You are free to withdraw at any time, without giving a reason (this will not affect the standard of care you receive).

What will happen to me if I take part?

10 ml of sterile normal phosphate buffered saline will be offered to you to rinse your mouth with. You are expected to rinse for 60 seconds in the presence of the researcher. This procedure is totally painless and no bleeding or tissue damage will ensue afterwards. Collected oral rinse samples will then be sent for microbiological study in the laboratory to culture different bacteria that are commonly found in the mouth. You will be referred to the appropriate department within our faculty in case any dental or oral disease that needs treatment is detected.

Participating in this study will cost you nothing; in fact it might save you money by the early detection of any dental or oral lesions which makes treatment easier and cheaper.

What will I have to do?

For the purposes of this study, nothing more is required from you. However, regular visits to the dentist in addition to sustained efforts to clean your teeth (by brushing and flossing) will always be encouraged if you want to stay healthy and keep your teeth in good shape.

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What are the possible disadvantages and risks of taking part?

No perceived disadvantages or risks are expected to result from taking part in this study.

What are the possible benefits of taking part?

We cannot promise the study will help you, but the information we get from the study will help to increase the understanding of the microbiology of oral fungal infections, gum disease and dental caries.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researcher who will do his best to answer your questions (contact number: 0798632238).

If you remain unhappy and wish to complain formally, you can do this through Professor LXG Stephen, diagnostic cluster chairperson, Faculty of Dentistry, University of Western Cape.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital will have your name and address removed so that you cannot be recognized.

How your data will be collected?

Samples collected from you as a participant will be given a code known only to the researcher before being sent for laboratory examination. A master list identifying participants to the research codes data will be held on a password protected computer accessed only by the researcher. Hard paper will be stored in a locked cabinet, within locked office, accessed only by the researcher. Electronic data will be stored on a password protected computer known only by the researcher. Your data will be accessible only to authorized persons such as researchers within the team, supervisors, sponsors and for monitoring the quality, regulatory authorities /R&D audit. Your data will be retained for a period of 3 years before it will be disposed of securely.

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What will happen if I don't carry on with the study?

If you withdraw from the study we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What will happen to the results of the research study?

The results of this research study will be submitted as a thesis for a master degree, and if the degree is approved by the university senate, I intend to publish these results in dental research journals. These results can be made available to you by sending it via e-mails if you wish to be notified by the outcome of the study. We confirm again that you will not be identified in any report/publication unless you have given your personal consent.

Who is organizing or sponsoring the research?

The University of the Western Cape represented by two departments –the Department of Oral Medicine and Periodontics, and the Department of Medical Biosciences- will be organizing and sponsoring this research project.

Further information and contact details:

1. General information can be found at medical research websites like www.pubmed.gov or www.cdc.gov

2. For specific information about this research project, you are welcome to contact me at this e-mail address 3115947@uwc.ac.za

This study has been ethically reviewed and approved by the UWC Senate Biomedical Research Ethics Committee (approval number_____).

Appendix 2

Informed Consent

I, (Name…….) have been informed about the study entitled the antimicrobial efficacy of 3 chlorhexidine mouth rinses: an in-vitro analysis, by Dr.BasheerAbdalrahman.

I understand the purpose and procedures of the study.

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at cell phone number 0798632238 or via e-mail 3115947@uwc.ac.za

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If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

DENTISTRY RESEARCH ETHICS COMMMITTEE

Research Office, Tygerberg Campus

Francie van Zyl Drive

Private Bag X1

Tygerberg

7505

Cape Town, SOUTH AFRICA

Tel: 27 21 937 3095 - Fax: 27 21 931 2287

Email: suenaidoo@uwc.ac.za

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

(Where applicable)

_____ _____

Signature of Translator Date

(Where applicable)

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Appendix 3

Data Capturing Tables

Table (A): Readings from oral rinse cultures
*FCLT: Facultative aerobic culture, AnO2: Strictly Anaerobic culture.
** CHX1: Pure CHX 0.2%, CHX2: Pure CHX 0.12%, CHX3: Corsodyl, CHX4: Curasept, CHX5: Paroex

	Oral rinse 1		Oral rinse 2		Oral rinse 3		Oral rinse 4		Oral rinse 5		Oral rinse 6		Oral rinse 7	
	FCLT	AnO2	FCLT	AnO2	FCLT	AnO2	FCLT	AnO2	FCLT	AnO2	FCLT	AnO2	FCLT	AnO2
CHX1														
CHX2														
CHX3														
CHX4														
CHX5														

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Table (B): Readings from pure *Streptococcus mutans* cultures * CHX1: Pure CHX 0.2%, CHX2: Pure CHX 0.12%, CHX3: Corsodyl, CHX4: Curasept, CHX5: Paroex							
	S. mutans 1	*S. mutans* 2	*S. mutans* 3	*S. mutans* 4	*S. mutans* 5	*S. mutans* 6	*S. mutans* 7
CHX1							
CHX2							
CHX3							
CHX4							
CHX5							

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Table (C): Readings from pure *Candida albicans* cultures * CHX1: Pure CHX 0.2%, CHX2: Pure CHX 0.12%, CHX3: Corsodyl, CHX4: Curasept, CHX5: Paroex							
	C. albicans 1	*C. albicans* 2	*C. albicans* 3	*C. albicans* 4	*C. albicans* 5	*C. albicans* 6	*C. albicans* 7
CHX1							
CHX2							
CHX3							
CHX4							
CHX5							

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