ANTIMICROBIAL EFFICACY OF NON-CHLORHEXIDINE NON-ALCOHOL CONTAINING MOUTHRINSES

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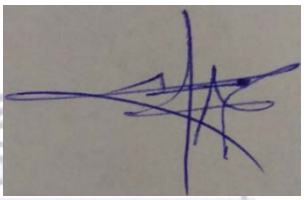
DECLARATION

I hereby declare that this mini thesis entitled Antimicrobial efficacy of non- chlorhexidine non-alcohol containing mouthrinses is my own work and that I have not previously submitted it at any university for a degree or examination. All sources that I have quoted have been indicated and duly acknowledged by means of referencing.



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ABSTRACT

Aim: to compare antimicrobial efficacy of three different non-chlorhexidine non-alcohol containing mouthrinses.

Objectives: to test antimicrobial efficacy of Colgate total®, Biobalance mouthwash® and Listerine Zero® against *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans*, *Enterococcus faecalis* and the aerobic and facultative anaerobic organisms cultured from the collected oral saliva.

Materials and methods: fourteen saliva samples were collected from staff members who fit the inclusion criteria along with fourteen pure cultures of each of the tested microorganisms. All samples were cultured in agar plates. Four sterile, 5mm discs were used for each plate, each representing one of the tested mouthrinses. Each disc was immersed for one minute and then deposited on sterile gauze to remove excess fluid. Then plates were incubated for 24 hours at 37˚ Celsius. Inhibition zones created around the discs were measured using an electronic caliber.

Results: most of the tested mouthrinses showed antimicrobial efficacy against tested microorganism. Differences between them were statistically significant (p. value =0.0001). The order in terms of antimicrobial efficacy was Colgate total®> Biobalance mouthwash® > Listerine Zero®.

Conclusion: Colgate total® showed antimicrobial efficacy against all tested microorganisms. Whereas Biobalance mouthwash® failed to obtain significant antimicrobial efficacy against *Enterococcus faecalis*. Listerine Zero® failed to accomplish significant results, the reason behind that is unknown.

Keywords: Antimicrobial – efficacy – non-chlorhexidine – non-alcohol – mouthrinses.

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CHAPTER 1

Introduction and Literature review

1.1 Introduction:

It is generally accepted that adherence of plaque to hard and soft tissues of the mouth can cause gingivitis and dental caries. Several approaches have been used to reduce dental plaque (Gaffar *et al*, 1997).

Most adults who stick to the two minutes tooth brushing only remove 50% of the accumulated plaque. Therefore the use of mouthrinses and other chemical plaque control methods is justifiable (Van der Weijden *et al*, 2008). Most mouthrinses used to control plaque mainly consist of an active ingredient or an antiseptic agent, ethanol (as a solvent) or water in addition to surfactants, humectants and flavorings (Addy and Moran, 2008). The most popular active ingredients are chlorhexidine, essential oils, cetylpyridinium chloride and the newly introduced carbohydrate fulvic acid (Addy and Moran, 2008). These ingredients can have bacteriocidal or bacteriostatic mechanisms of action (Addy and Moran, 2008).

Mouthrinses have a wide range of uses, mainly as an adjuvant method to mechanical tooth brushing. It has been proven that some mouthrinses reduce halitosis via reduction of volatile sulphur compounds (Carvalho *et al*, 2004). Mouthrinses are often used in hospital settings as a supplement for mechanical tooth brushing for example with unconscious or disabled patient due to physical or mental inability or as a post-operative care measure. However, some of these mouthwashes have adverse effects either due to the active ingredient itself e.g. chlorhexidine or due to one of additive materials such as alcohol (Eldridge *et al*, 1998).

In this study the antimicrobial efficacy of non-chlorhexidine non-alcohol (NC-NA) containing mouthrinses was tested against various oral microorganisms in *vitro*.

1.2 Literature review

1.2.1 Chlorhexidine

Chlorhexidine is a chemotherapeutic agent which is widely used as an adjunct to mechanical plaque control. It reduces plaque accumulation by 60 % (Bascones *et al*, 2005) and is found in three different formulations; digluconate, acetate and hydrochloride (Addy and Moran, 2008).

The chemical structure of chlorhexidine is complex, made up of two 4- chlorophenyl rings and two biguanide groups linked by a central hexamethyline chain. This makes it more basic in nature as it gains bicatonic charge which has high capacity for anionic elements (Bascones *et al*, 2005).

Mechanism of action and clinical efficacy

Chlorhexidine acts as a bacteriostatic agent by increasing membranous permeability of microorganisms, thus altering potassium intracellular concentration (Bascones *et al*, 2005). It can also act through inhibiting proteolytic and glycocidic enzymes (Addy and Moran 2008). At higher concentrations, chlorhexidine can be bacteriocidal by inducing cytoplasmic precipitation which eventually leads to cell death.

Due to its chemical structure chlorhexidine has the ability to be adsorbed by both hard and soft tissues to be slowly released over a period of 8 -12 hours. This is known as substantivity (Bascones *et al*, 2005).Thus it is the most clinically effective mouthrinse currently available (Bascones *et al*, 2005).

In vitro, it was proven that chlorhexidine is effective against Gram-positive and Gram-negative microorganisms including aerobes and anaerobes, together with fungi. However, the gram negative anaerobes were decreased to a lesser degree. No resistance was observed against chlorohexidine despite long periods of usage extending up to two years (Addy and Moran 2008).

Clinically chlorhexidine has two popular concentrations: 0.2 % and 0.12%, (Rath and Singh 2013). A comparative study that was conducted between the two concentrations concluded that both have optimum clinical results. However, the adverse effects of chlorhexidine are dose and

concentration dependent. Therefore, the 0.12% concentration was recommended because of its better patient compliance (Rath and Singh 2013).

It was reported that the use of 0.2 % mouthrinse shows 43% reduction in volatile sulphur compounds (VSC) values and 50% reduction in organoleptic halitosis ratings .De Boever and Loesche as cited by Perry *et al*, 2009 reported 73% VSC, 69% mouth odor and 78% tongue odor reductions when 0.12% concentration was used.

Alcohol is added to some chlorhexidine preparations to act as an antiseptic and/ or as a solvent to carry other ingredients. (Eldridge *et al*, 1998; Borrajo *et al*, 2002).

Adverse effects

Although different concentrations of chlorhexidine are available; it was found that the adverse effects are dose dependent (Cancro *et al*, 1974) and concentration dependent (Greenstein *et al*, 1986). Chlorhexidine has localized side effects which may appear due to prolonged usage. This is mainly reflected as brown staining of teeth, tongue and transient impairment of taste (Perry *et al*, 2010) and increased calculus formation (Rath *et al*, 2013). Allergies (type 1 hypersensitivity reaction) and allergic contact dermatitis and stomatitis (type 4 hypersensitivity reaction) were also reported (Pemberton and Gibson 2012).

1.2.2Essential oils based mouthrinses

Usually essential oils based mouthrinses include thymol (0.064 %), eucalyptol (0.092 %), menthol (0.042 %), and methylsalicylate (0.060 %) (Perry *et al*, 2009; Charles *et al*, 2012). Alcohol is added to essential oils preparations in a certain concentration that dissolves the essential oils but not enough to act as an antiseptic (Cortelli *et al*, 2013).

The antiplaque and the antigingivitis effect of essential oils were reported by several clinical studies (Amini *et al*, 2009). They reduce plaque by 20% – 35% and gingivitis by 25%- 35% (Perry *et al*, 2010). Another study was conducted on the efficacy of essential oils and showed greater plaque reduction i.e. 56% and 35% gingivitis reduction when used alone without mechanical control (Amini *et al*, 2009).

Essential oils based mouthrinses show immediate penetration of the biofilm as well as substantivity that lasted for 7 hours after application (Quintas *et al*, 2014).

A newly introduced Listerine Zero® (Johnson and Johnson Ltd.) mouthrinse is an essential oil based mouthrinse that does not contain alcohol (Cortelli *et al*, 2013). According to the manufacturers, they were able to develop new compounds that could stabilize the essential oils without the need for alcohol (Charles *et al*, 2012). A two weeks randomized control study was carried out to test the efficacy of Listerine Zero®; the results showed that there was a reduction by 23.9% and 10.4% in plaque and gingival indices respectively when compared to the control group (Charles *et al*, 2012). Another six month randomized clinical trial was carried out by Cortelli *et al*, 2013 where Listerine Zero® was compared to alcohol free cetylpyridinium. The study showed that Listerine Zero® reduced plaque and gingivitis by 31.6% and 4.4%.

Adverse effects of using alcohol in both chlorhexidine and essential oils based mouthrinses

Eldridge *et al*, 1998 showed that mouthrinses that contained less than 10% of alcohol did not induce pain, but there was concern about using alcohol at more than 24% because of the potential increase in the risk of oral and pharyngeal cancer in patients who used it on a daily basis. In addition, high ethanol concentrations, if combined with low pH of a mouthrinse, can cause irritation of oral mucosa. The authors recommended that these be contraindicated it in patients with mucositis. Alcohol can be an aggravating factor to the side effects of oral and neck radiotherapy such as xerostomia, ulcerating gingivitis and tissue damage (Borrajo *et al*, 2002; Bascones *et al*, 2005). Moreover, it is also contraindicated in immunocompromised patients and chronic alcoholic patients as it can increase the intensity of the side effects of alcohol abuse (Bascones *et al*, 2005). Lemos-Junior and Villoria (2008) also reported that mouthwashes, which contained 26.9 % ethanol, could be lethal for children weighing up to 26 pounds if 5 to 10 ounces were ingested.

1.2.3Cetylpyridinium chloride

Cetylpyridinium chloride, also known as CPC is a quaternary ammonium compound with strong cationic properties, which readily binds to anionic particles (Cortesia et al, 2010). It acts primarily on Gram-positive bacteria and yeasts but some authors claim that it is antimicrobial against Gram-negative bacteria as well (Kang *et al*, 2012).

CPC mouthrinse acts via increasing membrane permeability and decreasing cellular adherence of microorganisms (Kang *et al*, 2012). According to Amini *et al*, (2009) in a six-month trial, it had

24% antigingivitis and antiplaque effects. CPC efficacy may be increased if combined with chlorhexidine (Kang *et al* 2012).

CPC can be found as alcohol combined as well as alcohol-free mouthrinse formulations. However, there is no significant difference between the efficacies of the two formulas as antiplaque being 6.2% and 9.4% or as antigingivitis being 6.1% and 5.8% respectively (Amini *et al*, 2009). It was found that essential oils based mouthrinses have an *in vitro* antimicrobial effect two times greater than 0.05% CPC whether it is combined with alcohol or not (Amini *et al* 2009).

1.2.4 Carbohydrate derived fulvic acid 0.5% (CHD-FA)

CHD-FA is a heat stable low molecular weight, water soluble, cationic, colloidal material with proposed therapeutic properties (Sherry *et al*, 2012). CHD-FA is an organic acid with proven efficacy against *Candida albicans* biofilms (Sherry *et al*, 2012) as well as bacterial oral films. It was developed in order to overcome the side effects of chlorhexidine and to modulate the immune response of the host (Sherry *et al*, 2013).

CHD-FA mouthrinse has both bactericidal and bacteriostatic properties. It is active against various oral pathogenic organisms including *Aggregabacter actinomycetemcomitans*, *Streptococcus mutans* and *mitis*, *Enterococcus faecalis*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* (Sherry *et al*, 2013). When compared to chlorhexidine, none of the tested microorganisms used showed more sensitivity or resistance to either compound. However CHD-FA showed more rapid antimicrobial activity as after thirty minutes there was a reduction in the polymicrobial biofilm by 90% (Sherry *et al*, 2013).

Moreover, CHD - FA displayed no toxicity when tested on rats and humans at pH of 7.0 and had anti-inflammatory and wound healing properties (Sherry *et al*, 2013, Grandy *et al*, 2012). Grandy *et al*, (2012) also reported that it was safe up to 40 ml of mouthrinse twice daily for a week.

According to the available literature, there is insufficient data on the efficacy of NC-NA containing mouthrinses.

1.3 Aim and Objectives

1.3.1 Aim:

To compare the antimicrobial efficacy of three different NC-NA containing mouth rinses OTC, available locally.

1.3.2 Objectives:

To test the antimicrobial efficacy of:

1. Resmed chlorhexidine gluconate® (Resmed Healthcare. South Africa).
2. Listerine Zero ® (Johnson and Johnson Ltd. South Africa).
3. Colgate total ® (Colgate Palmolive Ltd. Thailand).
4. Biobalance mouthwash® (Fulvicare Ltd. South Africa).

Against:

1. *Staphylococcus aureus* (ATCC25923).
2. *Streptococcus mutans* (ATCC 25175).
3. *Candida albicans* (ATCC 36810).
4. *Enterococcus faecalis* (ATCC 29212).
5. Facultative anaerobes prepared from oral rinse samples.

CHAPTER TWO

MATERIALS AND METHODES

2.1 Study design:

An in-*vitro* analytical study of an exploratory nature was carried out.

2.2 Study site:

Oral and Dental Research Institute laboratory, Faculty of Dentistry, University of the Western Cape.

2.3 Study participants:

Oral rinse samples were randomly collected from 14 staff members from the University of the Western Cape, Faculty of Dentistry. All enrolled adult individuals were dentate or partially dentate and all were orally and systematically healthy. Exclusion criteria included dentulous individuals, smokers and snuff dippers and those who have used antibiotics, immunosuppressive or chemotoxic drugs during the past three months prior to sample collection.

2.4 Collection and preparation of oral rinse samples:

Participants were asked to rinse thoroughly for 60 seconds with 10 ml of sterile saline provided in a universal container and to return the rinse into the container (Samaranayake, 1986).

2.5 Measurements of bacterial densities in oral rinse suspensions:

The samples from participants were standardized using the McFarland standard scale for measuring bacterial densities in suspensions. The turbidity of the McFarland standard and the oral rinse suspension was compared by holding the oral rinse and McFarland Standard tubes up against the black and white bars printed on enclosed cards. Standardization was reached when the turbidity of the fluid inside the two suspensions matched the McFarland standard 1 turbidity tube (corresponding approximately to 3×10^8 CFU/ml).

The McFarland Standard tubes contain latex particles suspended in a liquid buffer that are adjusted to an acceptable transmission range using a spectrophotometer, at a wave length of

either 600 or 625 nm. Bacterial suspensions with similar turbidity to a particular McFarland Standard are expected to produce approximate cell count densities. This method is used in a variety of identification or susceptibility kits (Borges *et al*, 2010).

2.6 Preparation of oral rinse cultures:

One hundred µl (100 µl) of each suspension was inoculated onto standard Brain Heart Infusion agar (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. Then the plates were incubated for 24 hours at 37℃.

2.7 Preparation of pure cultures:

Cultures of *Staphylococcus aureus* (ATCC 25923), *Streptococcus mutans* (ATCC 25175), *Candida albicans* (ATCC 36810) *and Enterococcus faecalis* (ATCC 29212) were tested because they have a well-known role in the development of dental caries, candidiasis and primary endodontic lesions respectively. Cultures were incubated for 24 hours at 37ºC. A separate inoculum from each culture was prepared by suspension in saline using the direct colony suspension method. The suspension of the pure cultures was also standardized with the McFarland Standard 1 as described above.

2.8 Disc infusion test to measure inhibition zones:

70 agar plates were divided into 5 groups as follows:

- Group A: 14 aerobic and facultative anaerobic bacteria cultured plates prepared from oral rinse samples.
- Group B: 14 plates of pure cultures for *Staphylococcus aureus* bacteria.
- Group C: 14 plates of pure cultures for *Streptococcus mutans* bacteria.
- Group D: 14 plates of pure cultures for the fungus *Candida albicans.*
- Group E: 14 plates of pure cultures for *Enterococcus faecalis* bacteria.

2.9 Mouthrinses used in this study

1. Listerine Zero® mouthrinse: essential oils with sodium fluoride (Resmed Healthcare. South Africa).

2. Colgate total® mouthrinse: sodium fluoride 0.05% (225 ppm F) and cetylpyridinium chloride 0.075% (Johnson and Johnson Ltd. South Africa).

3. Biobalance mouthwash®: CHD-FA (20%) (Colgate Palmolive Ltd. Thailand).

4. Resmed chlorhexidine gluconate 0.2% mouthrinse®. (Fulvicare Ltd. South Africa), as a positive control.

The next step was the insertion of the absorbent paper disks, 4 sterile, with 5 mm-diameter, obtained by patterned perforation of coffee filter paper for each of the 70 agar plates. All disks were immersed in equal time (1 min) in respective substances and then in sequence, deposited neatly on sterile gauze to remove liquid excess (Borges *et al*, 2010). The antibacterial effect of each mouthrinse product was measured in terms of the dimensions of the bacterial growth inhibition zone around the disks that occurred within 24 hours of incubation. (Figure 1).

Inhibition zones were measured thrice from various points and angles using a digital caliber. (Figure 2).

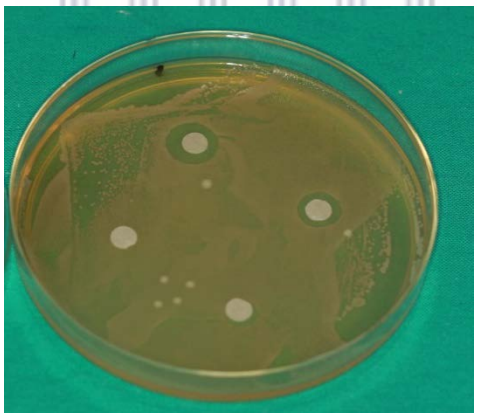

Figure 1: inhibition zones around the discs

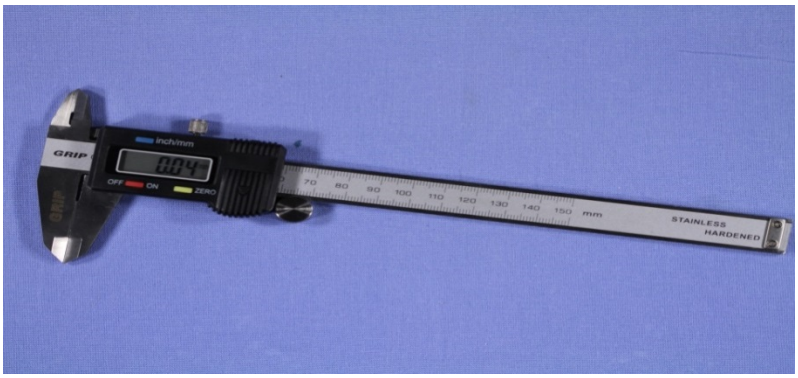

Figure 2: digital caliber used in measurement

2.10 Data analysis:

The data was categorized and coded and then entered into a data capture sheet using Microsoft Excel sheet (Microsoft Corporation®, Redmond, Washington). The database was imported into Social Package of Statistical Analysis (SPSS-IBM Corporation®, Armonk, New York) to perform statistical analysis. A statistician was consulted and the results were presented appropriately in the form of frequency tables and graphs.

2.11 Ethical approval:

Ethical approval was obtained from the UWC, Faculty of Dentistry. Fourteen individual participants consent for specimen collection was obtained via written informed consent (Appendix 1, 2). 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.

CHAPTER 3

RESULTS

The results obtained from each type of bacterial suspension that was co- cultured with each of the four types of mouthrinses in the fourteen different perti dishes and the mean measurement of the clear zone that was calculated are presented in Table 1.

The results obtained from the saliva samples showed that the mean measurement of the clear zone was significantly higher when using Resmed chlorhexidine compared to other mouthrinses. Colgate Total® displayed the highest measurement. Whereas, no significant difference between Listerine Zero® and Biobalance mouthwash® was observed (Table 3.2, Fig 3.1). Similar results were obtained when *Enterococcus faecalis* was tested with each mouthrinse (Table 3.3, Fig. 3.2).

Table 1: Mean measurement of clear zone in mm of the four types of mouthrinses co-cultured with sessile aerobes and facultative anaerobes obtained from saliva, *S. aureus, S. mutans, C. albicans and E. faecalis*

	Chlorhexidine	Colgate total	Listerine Zero	Biobalance mouthwash
Oral flora obtained from saliva	10.27	7.95	0	0.57
Staphylococcus aureus	19.55	13.6	1.23	7.10
Streptococcus mutans	22.02	17.10	0	9.30
Candida albicans	12.20	10.10	0	2.35
Enterococcus faecalis	11.08	9.4	0	0

Table 2: The Mean measurement of the clear zone of sessile aerobes and facultative anaerobes obtained from saliva co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®

	Mean±SD	***P*-value**
Resmed chlorhexidine 0.2%®	10.27 ± 4.65^{a}	0.0001
Colgate Total®	7.95 ± 2.42^{b}	
Listerine Zero®	0^{c}	
Biobalance mouthwash®	0.57 ± 2.14^{c}	

(A, b): Means different superscripts are significant ($p<0.05$)

Table 3: The Mean measurement of the clear zone of *Enterococcus faecalis* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®

	Mean±SD	***P*-value**
Resmed chlorhexidine 0.2%®	11.08 ± 0.47^{a}	0.0001
Colgate Total®	9.40 ± 0.70^{b}	
Listerine Zero®	0^{c}	
Biobalance mouthwash®	0^{c}	

(A, b): Means different superscripts are significant ($p<0.05$)

Results from the *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* bacterial suspensions revealed that the mean measurement of the clear zone was significantly higher when using Chlorhexidine compared to other mouthwashes. Colgate total® showed higher measurement than both Listerine Zero® and Biobalance mouthwash® the mean clear zone measurement was significantly higher with Listerine Zero® than Biobalance mouthwash® (Tables 3.4, 3.5, 3.6, Figs 3.3, 3.4, 3.5) respectively.

Table 4: The Mean measurement of the clear zone of ***Staphylococcus aureus*** suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®

	Mean±SD	**P-value**
Resmed chlorhexidine 0.2%®	19.55±3.17[a]	0.0001
Colgate Total®	13.60±4.49[b]	
Listerine Zero®	1.23±4.59[c]	
Biobalance mouthwash®	7.09±5.94[d]	

(A, b): Means different superscripts are significant ($p<0.05$)

Table 5: The mean measurement of the clear zone of *Streptococcus mutans* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®

	Mean±SD	**P-value**
Resmed chlorhexidine 0.2%®	22.02±1.45[a]	0.0001
Colgate Total®	17.10±2.41[b]	
Listerine Zero®	0[c]	
Biobalance mouthwash®	9.31±1.62[d]	

(A, b): Means different superscripts are significant ($p<0.05$)

Table 6: The Mean measurement of the clear zone of ***Candida albicans*** suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®

	Mean±SD	***P*-value**
Resmed chlorhexidine 0.2%®	12.20±0.96[a]	0.0001
Colgate Total®	10.10±1.94[b]	
Listerine Zero®	0[c]	
Biobalance mouthwash®	2.35±3.28[d]	

(A, b): Means different superscripts are significant ($p<0.05$)

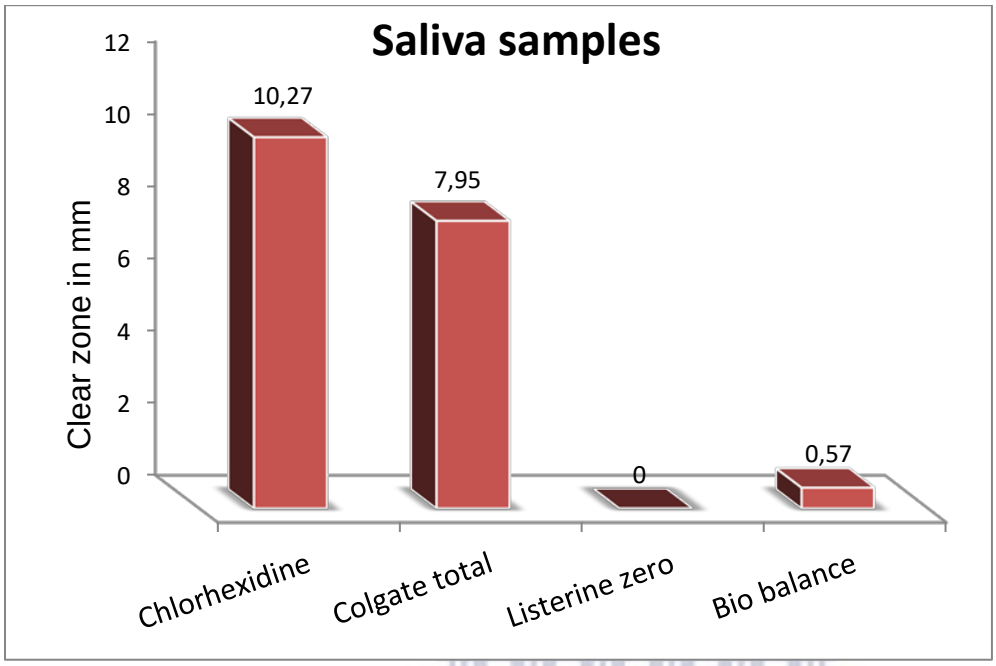

Figure 3: The difference in the mean measurement of the clear zone of sessile aerobes and facultative anaerobes obtained from Saliva co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®.

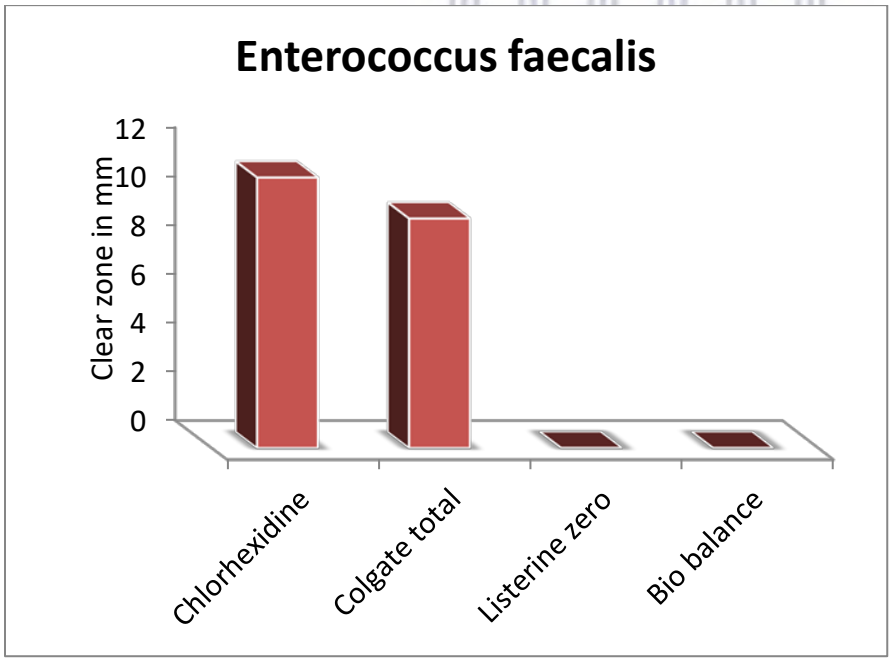

Figure 4: The difference in the mean measurement of the clear zone of *Enterococcus faecalis* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®.

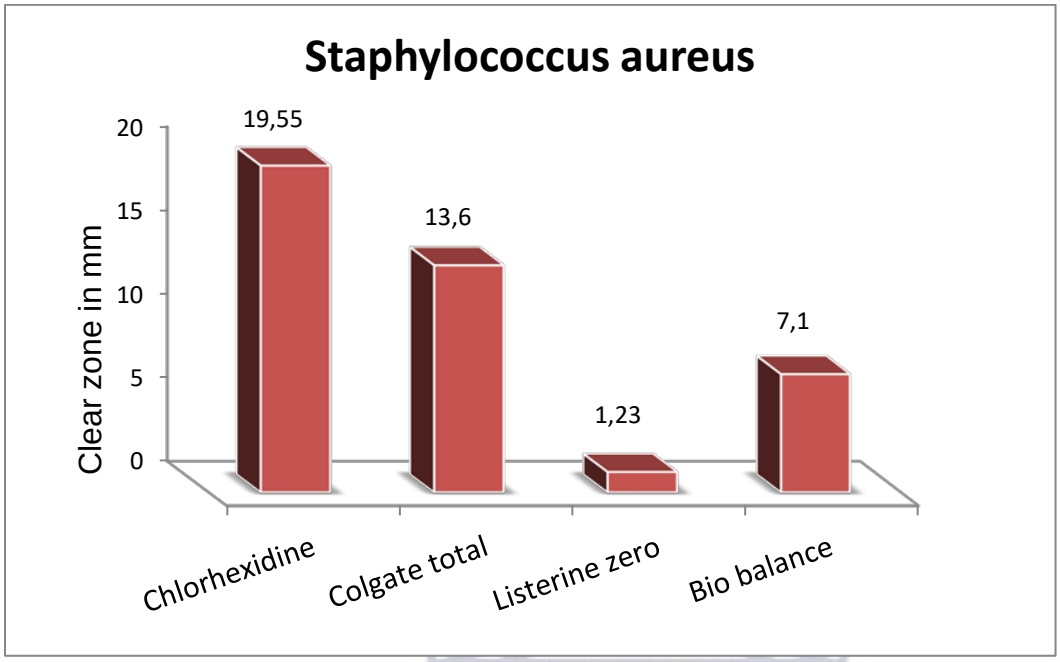

Figure 5: The difference in the mean measurement of the clear zone of *Staphylococcus aureus* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®.

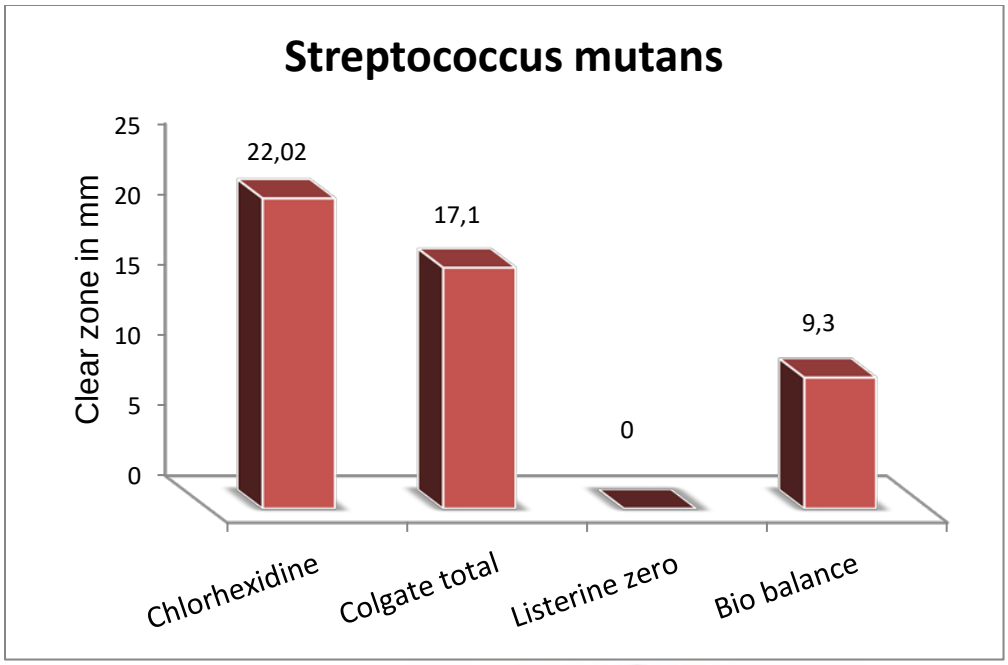

Figure 6: The difference in the mean measurement of the clear zone of *Streptococcus mutans* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®.

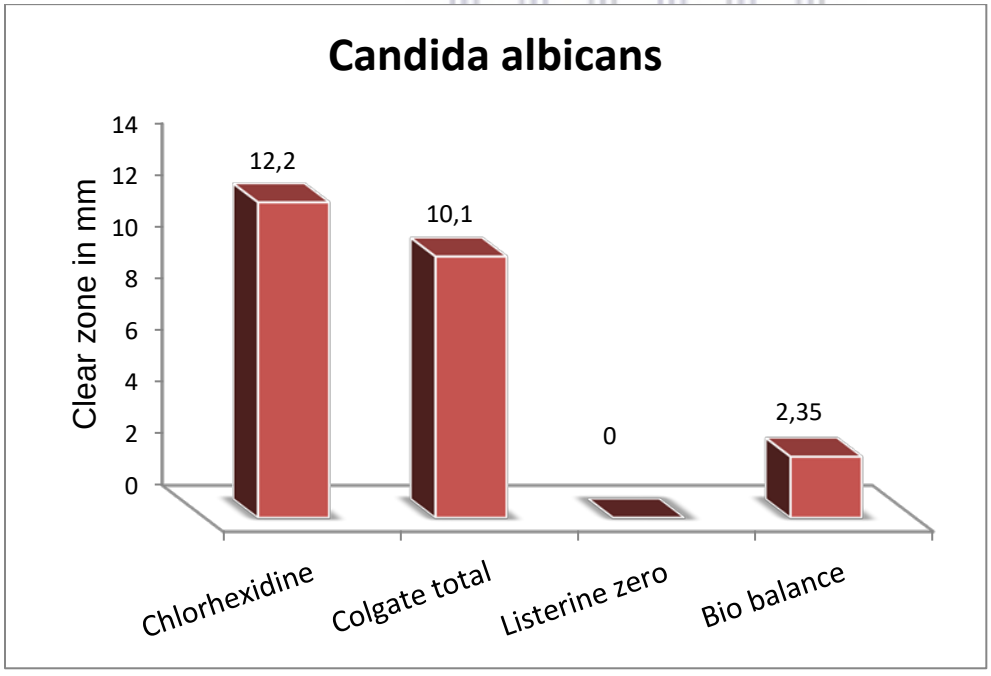

Figure 7: The difference in the mean measurement of the clear zone of *Candida albicans* suspension co-cultured with Resmed chlorhexidine 0.2%®, Colgate Total®, Listerine Zero® and Biobalance mouthwash®.

CHAPTER FOUR

DISCUSSION

In this *in-vitro* study the antimicrobial efficacy of three different mouthrinses were tested against salivary oral flora and pure cultures of selected bacteria. None of these mouthrinses contained chlorhexidine or alcohol. The organisms used were obtained from two different sources i.e. standard laboratory strains as well as oral salivary rinse samples. The laboratory strains were selected based on their association with oral diseases and included *Streptococcus mutans*, *Staphylococcus aureus, Candida albicans* and *Enterococcus faecalis.*

Aerobes and the facultative anaerobes were cultured from saliva that was collected from 14 staff members of Faculty of Dentistry, University of the Western Cape. This method was selected based on previous studies by Samaranayake (1986) that indicated that salivary rinse samples have the ability to represent all microorganisms present in the oral cavity including periodontal pathogens present in dental pockets. This allowed the investigator to determine the efficacy of the mouthrinses against oral microorganisms that originated from both a planktonic as well as a sessile state from the oral cavity.

Based on the results obtained, the test mouthrinses showed different degrees of antimicrobial efficacy against the cultured microorganisms (Table 3.1). These differences were statistically significant (*p.* value = 0.0001). The order in terms of antimicrobial efficacy against *Streptococcus mutans, Staphylococcus aureus, Candida albicans, Enterococcus faecalis* and aerobes and facultative an aerobes obtained from saliva is Colgate Total® > Biobalance mouthwash® > Listerine Zero®.

Colgate Total® showed the greatest degree of antimicrobial efficacy, this antimicrobial dominance was displayed across the range of organisms tested including *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans, Enterococcus faecalis* and the aerobic and facultative anaerobic organisms cultured from the collected oral saliva. The reason for the antimicrobial

efficacy of Colgate Total® seen in this study is not clear, but may be due to its active ingredient cetylpyridinium chloride (CPC). CPC has a broad antimicrobial spectrum largely based on its cationic chemical structure. This cationic property allows it to bind to anionic bacterial cell walls leading to disruption of the cellular membrane, leakage of the intracellular components, and eventual cell death (Kang *et al*, 2015; Almas et al, 2005). The efficacy of CPC against *Candida albicans* has been demonstrated in several *in-vitro* and *in-vivo* studies with the present study showing similar efficacy as was previously mentioned in the literature. The exact mechanism of action against *Candida albicans* is poorly understood but it is thought to be related to an interaction with the fungal cell wall (Pizzo *et al*, 2001). Recent research also indicates that CPC has the ability to reduce the attachment of *Candida albicans* to epithelial cell walls (Pizzo *et al*, 2001).

Biobalance mouthwash® was the second most effective mouthrinse in terms of antimicrobial efficacy. It has CHD-FA as an active ingredient which acts via disrupting the bacterial and fungal cellular membranes leading to lysis and eventually death. (Sherry *et al*, 2012; Sherry *et al*, 2013).

According to Sherry *et al*, 2013 CHD-FA has a broad spectrum of antimicrobial activity against oral pathogens in both planktonic and sessile forms as well as laboratory designed biofilms. However, in this study mouthrinse containing CHD-FA failed to obtain significant antimicrobial efficacy against *Enterococcus faecalis* colonies, the reason behind this is unknown. On the other hand the same mouthrinse showed significant amount of reduction in other microorganisms tested in the study. It is worth mentioning that the concentration of CHD-FA used in this study was 20% which is higher concentration than that used by Sherry *et al*, 2013.Whether this had any significance is unknown.

Listerine Zero® utilizes essential oils as active ingredients. They act via penetrating the biofilm and disrupting the intercellular bonds of plaque forming microorganisms (Quintas *et al*, 2014). In this study Listerine Zero® failed to show any significant antimicrobial efficacy against microorganisms tested. A 6 month randomized control study was carried out Cortelli et al to compare the antimicrobial efficacy of alcohol free essential oil with CPC containing mouthrinses in terms of reduction of plaque and gingivitis. They found that alcohol free essential oil containing mouthrinse reduced plaque and gingivitis by 16.1% and 6.7% respectively. While

CPC reduced plaque and gingivitis by 6.9% and 5.1% respectively (Cortelli *et al*, 2013). The variation in terms of results between Cortelli *et al*, 2013 and this study might be due to the absence of intercellular bonds which is significant to the plaque biofilm.

Absence of alcohol might be another reason why Listerine Zero® did not show significant antimicrobial efficacy against microorganisms tested in this study. It is worth mentioning that alcohol is a major constituent in other Listerine® mouthrinses.

Difference in concentrations of active ingredients and other additive materials that may interfere with the chemical formula of active ingredients are considerable factors that might contribute to these findings.

CHAPTER FIVE

Conclusions

Colgate total® showed antimicrobial efficacy against all microorganisms tested whereas Biobalance mouthwash® failed to show significant antimicrobial efficacy against *Enterococcus faecalis*, though it was successful in showing significant results against other microorganisms tested in the study. Listerine Zero® failed to accomplish significant antimicrobial efficacy against tested microorganisms and the reasons behind that is unknown.

Results of this study can act as a platform for further clinical investigations regarding the use of NC-NA containing mouthrinses as a novelty treatment for dental patients.

CHAPTER 6

Limitations

Microorganisms suspended from oral rinse samples do not possess the biofilmatic properties of dental plaque, which may have had interference with the mechanism of action of some active ingredients of mouthrinses used in the study. Plaque defensive mechanisms against active ingredients in mouthrinses were not counted for in this study. Finally oral flora obtained from oral rinse samples (aerobes and facultative anaerobes) were dealt with collectively, sensitivity of specific bacterial colonies was not tested.

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Appendix 1

Information Sheet

Efficacy of non-chlorhexidine non- alcohol containing mouth rinses: an in-vitro analysis

I am Dr. A. Abdelhadi, 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 the antimicrobial efficacy of three different NC-NA containing mouthwashes. 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 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.

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.

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: 0783125655).

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.

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 3412536@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 three non-chlorhexidine non-alcohol containing mouth rinses: an in-vitro analysis, by Dr. A. Abdelhadi.

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 (078)312-5655 or via e-mail 3412536@myuwc.ac.za

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

_____ _____

Signature of Participant DateReferences