

Full Length Research Article

EFFICACY OF DIFFERENT BRANDS OF MOUTH RINSES ON ORAL BACTERIAL LOAD COUNT IN HEALTHY ADULTS

O.O. AKANDE^{*1, 3} A.R.A. ALADA¹, G.A. ADERINOKUN² AND A. O. IGE.¹

Departments of ¹Physiology, ²Preventive Dentistry and ³Oral Pathology,
 College of Medicine University of Ibadan, Ibadan

A laboratory test was used to assess the inhibition of growth of oral bacteria by three modern commercial mouthrinses containing either cetylpyridinium chloride, CPC (Macleans^(R)), phenolic compound (Colgate Plax^(R)) or glycerin/triclosan (Listerine^(R)); which were used unsupervised in a placebo-controlled study involving fortyfive healthy adults. Subjects were divided into 3 groups and their saliva samples were assessed for microbial counts at the beginning and the end of an eight-week period during which they rinsed with 10ml of mouthrinse for 15 seconds twice daily (morning and evening) in addition to their usual oral hygiene procedures. The results showed wide variations in their effectiveness: those containing CPC reduced oral microbial count significantly ($P < 0.05$) than formulations based on phenols or glycerin/triclosan. This concludes that twice daily use of CPC- containing mouthrinses reduce oral microbial load counts in healthy subjects when used as an adjunct to their normal oral hygiene procedures. This also suggests that inhibitory power of CPC is greater on oral microbes than other numerous functions of commercial mouthrinses.

Keywords: Mouthrinse [Colgate, Macleans, Listerin], Salivary flow, bacterial count

* Author for Correspondence

INTRODUCTION

Mouthwashes (mouthrinses) are solutions or liquids used to rinse the mouth for a number of purposes: (a) to remove or destroy bacteria (b) to act as an astringent (c) to deodorise and (d) to have a therapeutic effect by relieving infection or preventing dental caries¹. Constituents of mouthwashes include water (chief constituent); ethanol, dyes, surface active agents, zinc chloride /acetate, aluminium potassium sulphate (astringent); and phenolic compounds, quaternary ammonium compounds and essential oils such as oil of peppermint (as antibacterial agents) among others¹. Mouthwashes also provide a safe, effective chemical means of reducing or eliminating plaque accumulation (Combe, 1980; Ashley et al, 1984). A number of chemical agents are currently available in the market and are designed to assist individuals in their efforts to achieve and maintain oral health. While many agents are commercially available, the relative therapeutic benefits of most are not clearly defined. Kornman (1986) has suggested an organizational frame-work which allows classification of these topical

anti-microbial into one of two categories or generations based on their pharmacological properties. First generation agents can kill bacteria on contact, but have limited abilities to exert an effect on the oral flora after expectoration (e.g. cetylpyridinium chloride and sanguinarine), second generation agents have an immediate antibacterial effect and more importantly, have a prolonged effect on the oral flora (e.g. chlorhexidine). Mouthwashes are manufactured in two forms: the wash and the "spray". For most individuals the wash is a simple and acceptable method for the delivery of topical medicaments into the oral cavity. Rinsing with a chlorhexidine mouthwash is arguably the most effective chemical method to date of controlling plaque accumulation (Kalaga et al, 1989) Based on the original study (Addy, 1988) the most common regimen of use has been twice daily rinsing with 10ml of a 0.2% chlorhexidine solution (Kalaga et al, 1989; Addy, 1988; Jenkins et al, 1988). However, with the availability of more commercial mouthwashes (Babich and Babich, 1996; Leenstra, 1996) similar antiplaque effects have been reported (Heald et al, 1996; Goodson, 1996) with twice daily rinsing with

15ml or 10ml of the solution according to manufacturer's direction of use. Whereas previous studies (Kornman, 1986; Kalaga et al, 1989; Jenkins et al, 1988) have shown the ability of mouthwashes on plaque accumulation, plaque composition, either biochemical or microbiological, the possible effect of a mouthwash on bacterial load count in the mouth has received little or no attention in human studies in this environment. Based on this scanty information, the present study was designed to investigate the effectiveness of some commercially available mouthwashes (Macleans, Colgate plax and Listerine) on oral bacterial load count in healthy individuals.

MATERIALS AND METHODS

Forty five (45) adult (male 30 and female 15) students from the University of Ibadan community participated in the 8 weeks self-controlled study. To be accepted into the study, subjects (a) had to sign an informed consent, (b) must have full complement of the teeth; (c) must have a high standard of oral hygiene and gingival health, (d) had no relevant medical history, (e) were not receiving pharmacotherapy which might influence plaque accumulation or professional removal of plaque deposits and (f) were within (20-24) years old. Grounds for exclusion included pregnancy, debilitating disease, the need for antibiotic cover for dental treatment, steroid therapy or antibiotic medication either current or during the previous eight weeks.

Clinical Examination

The oral examinations were carried out in our Human Physiology laboratory with good illumination using mouth mirrors, and periodontal probes.

Subject grouping and saliva collection.

All the volunteers were divided into three groups: 15 subjects per group, each group having 10 males and 5 females. Subjects' saliva was collected early in the morning after normal oral hygienic procedures. Spitting method was employed in which the subjects were seated with head inclined forward so that the saliva will collect in the floor of the mouth from where it is spat into a 20 ml sterile specimen bottle for 5 minutes. The saliva samples collected in the first day for each group served as the control and the samples collected after 8 weeks of daily use of the grouped mouth rinse (Macleans),

(Colgate) and (Listerine) served as the experimental.

In each group, subjects rinsed the mouth first with 40ml water for 10 seconds after normal oral hygiene procedure [tooth brushing with toothpastes]. Subjects were asked to brush with the particular paste they were using before the start of the experiment. The saliva samples were sent to the Microbiology unit for culture and microbial count.

Mouth rinses:

The products evaluated in this study were Macleans (cetylpyridinium chloride, smithkline Beecham), Colgate Plax (glycerin, Colgate Palmolive) and Listerine (Phenolic essential oil, Warner- Lamber).

Sufficient rinse was provided for the eight- week period of the study and each subject was given detailed instruction in its use. They were asked to continue with their normal oral hygiene procedures but in addition, to rinse with 15ml mouth rinse for 10 seconds in the morning and evening and avoid the use of any other mouth rinses. Rinsing was to be carried out after tooth brushing, and eating and drinking were to be avoided for one hour after rinsing. Subjects were provided with a 15ml volume dispenser each and rinsing was otherwise unsupervised. In addition, subjects were questioned regarding possible use of antibiotics and any problems occurring during the study period.

P^H of each saliva sample was determined using the universal p^H paper and the salivary flow rate per minute for each subject was calculated.

Evaluation of microbial load count

Bacterial count (colony forming unit/ml c. f. u. /ml) in each sample was determined by culture and microscopy at the Department of Medical Microbiology and Parasitology, University College Hospital [UCH], Ibadan; before and after 8 weeks of daily use of the grouped mouth rinses.

Statistical Analysis

All statements of significance were based on alpha = 0.05, two- tailed test, and all percent reductions were calculated versus the control.

RESULTS

A total of forty five subjects (30 male and 15 female of age range 20 to 24 years) were recruited into the study and received the

baseline examination. None was excluded and all groups were well-matched at the beginning of the study with respect to age, sex, number of teeth, clinical condition and compliance with the rinsing programme. The subjects cooperated well, and none had any complaint throughout the study. The efficacy of 3 antimicrobial mouthrinses (Macleans, Colgate and Listerine) was evaluated after 8 weeks of continuous daily use. The control microbial load showed a preponderance of *staphylococcus aureus*, *Streptococcus pyogenes*, *streptococcus mutans*, *helicobacter pylori*, *Actinomyces viscosus*, *Candida albicans* and *Porphyromonas gingivalis*.

Table 1:

Mean Salivary pH, flow rate and bacterial load count in Healthy Adults using Macleans^(R) Mouthrinse

Saliva Sample	Control		Experimental	
	Male	female	Male	female
pH	7.0	6.0	8.0	8.0
Flow rate ml/min	0.84 ±.16	0.84 ±.01	1.3 ±.16	1.8 ±.01
Microbial Count [c.f.u/ml]	7.96 x10 ⁸ ± 0.2 x10 ²	6.92 x10 ⁸ ± 0.1 x10 ²	6.01 x10 ⁴ ± 0.2 x10 ^{2*}	4.52 x10 ⁴ ± 0.1 x10 ^{2*}

*5% level of significance.

Table 2:

Mean Salivary pH, flow rate and bacterial load count in Healthy Adults using Colgate^(R) Mouthrinse

Saliva Sample	Control		Experimental	
	Male	female	Male	female
pH	7.0	6.0	8.0	8.0
Flow rate ml/min	0.9 ±0.09	0.92 ±0.16	1.7 ±0.09	1.8 ±0.01
Microbial Count [c.f.u/ml]	8.64 x10 ⁸ ±0.4x10 ²	7.06 x10 ⁸ ±0.4x10 ²	7.06 x10 ⁸ ±0.2x10 ²	3.99 x10 ⁶ ±0.1x10 ²

The mean salivary pH, flow rate and microbial load count before (control) and after use of a mouth rinse designated to a particular group of Healthy adults are shown in Tables 1, 2 and 3. Shown in Table 1 are salivary pH, flow rate and microbial load count in subjects before and after 8 weeks use of Macleans mouth rinse. There were slight increases in the mean salivary pH and flow rate in contrast to a significant reduction in microbial count ($p < 0.05$) in both sexes. However, Tables 2 and 3 showed similar increases as well as reduced microbial load counts which are not statistically significant

with the use of Colgate and Listerine respectively.

Table 3:

Mean Salivary pH, flow rate and bacterial load count in Healthy Adults using Listerine^(R) Mouthrinse

Saliva Sample	Control		Experimental	
	Male	female	Male	female
pH	7.0	7.0	9.0	8.0
Flow rate ml/min	0.74± .09	0.76 ±.16	1.44 ±.16	1.44 ±.36
Microbial Count [c.f.u/ml]	8.86 x10 ⁸ ±0.13 x10 ²	7.14 x10 ⁸ ± 0.24 x10 ²	3.88 x10 ⁶ ±0.21 x10 ²	4.55 x10 ⁶ ±.13 x10 ²

DISCUSSION

This study was primarily designed to compare the efficacy of three mouth rinses containing cetylpyridinium, benzoic acid/eucalyptus oil or glycerine/triclosan, which are incorporated in Macleans, Listerine and Colgate respectively on oral bacterial load count.

In all the groups there were slight increases in salivary pH and flow rate after 8 weeks of mouth rinse application. This was in total disagreement with the work of Giertsen et al (1999) who observed no significant differences in their xylitol and fluoride containing mouthwashes. The increased salivary flow rate can be explained by the increased gustatory effects of these mouthrinses on oral salivary gland stimulation (Pitts et al, 1983).

In this study, we observed a significant reduction ($p < 0.05$) in microbial count in the Macleans group (Male 1.5% female 0.65%). This finding is in agreement with the report of Okuda et al (Okuda et al, 1998). The reduction may be due to the active principle contained in the Macleans mouth rinse which makes cetylpyridinium chloride (CPC), a quaternary ammonium compound one of the most potent active agents in a mouth rinse. This noteworthy finding in the Macleans group is consistent with previous investigations (Ashley et al, 1984; Grenby et al, 1984; Krammer et al, 1998; Jenkins et al, 1988).

In addition, clinical efficacy of CPC in reducing or eliminating plaque accumulation is well documented (Kalaga et al, 1989; Addy, 1988; Jenkins et al, 1988; Grenby and Saldanha, 1984). The mean microbial counts reduction in the colgate (male 0.07%, female 0.06%) and Listerine (male 0.10%, female

0.14%) groups are not statistically significant. This may be due to their suppressive action on oral microbes especially on the tongue and gingival crevices, but relatively little bactericidal powers. Also, they may not act as anti-microbial as claimed by the manufacturers but are good agents for general oral hygiene breath freshness, oral infections halitosis (Lochart, 1996; Lerardi et al, 1998), sore throat and ulcers (Fridh and Koch, 1999).

We conclude therefore, that the results of the present study showed that when used unsupervised as a part of regular oral hygiene and professional care, cetylpyridinium- containing mouthrinses (e.g. Macleans) provide significantly greater oral microbial reductions than do rinses containing either phenolic compounds or glycerin/triclosan to which Listerine and colgate belong respectively. However, where results are statistically significant, consideration should always be given to their clinical efficacy. We also conclude that with the unaffordable price hikes and the introduction of new brands of mouthrinses with improved formulations, it is up to the consumer to decide what he or she rates most highly in a mouthrinse: breath freshness, oral cleansing or an antiseptic action to control oral microorganisms and/ or pathogens and to choose accordingly.

REFERENCES

- Addy M (1986);** Chlorhexidine compared with other locally delivered antimicrobials. A short review. *J Clin Periodontol* 3: 957.
- Ashley FP., Skinner A, Jackson P, Woods A Wilson R.F (1984).** The effect of a 0.1% Cetylpyridinium Chloride Mouthrinse on Plaque and Gingivitis in Adults Subjects. *Br. Dent J.*; 157: 191-196.
- Babich H, Babich JP (1997).** Sodium lauryl sulfate and triclosan: in vitro cytotoxicity studies with gingival cells. *Toxicology Letters*; 91(3): 189-96.
- Combe EC (1980):** Notes on Dental Materials Churchill Livingstone. Edinburgh, London and New York: 268-269.
- Fridh G, Koch G (1999):** Effect of a mouthwash containing amyloglucosidase and glucose on recurrent aphthous ulcers in children and adolescents. *Sweedish Dent Journal.* (2-3): 49-57.
- Giertsen E, Emberland H. Scheie AA (1999):** Effects of mouthrinses with Xylitol and fluoride on dental plaque and saliva. *Caries Research.* 33 (1): 23-31.
- Goodson JM (1996):** Principles of Pharmacologic intervention. (Review) *J Clin Periodontol* 23 (3 Pt2): 268-72.
- Grenby TH, Saldanha MG (1984):** The Antimicrobial Activity of Modern Mouthwashes. *Br Dent J* ; 157: 239-242.
- Heald AE, Cox GM, Schell WA, Barlett JA (1996):** Perfect JR. Oropharyngeal yeast flora and fluconazole resistance in HIV- infected patients receiving long-term continous versus intermittent fluconazole therapy. *AIDS* ; 10 (3): 263-8
- Jenkins S, Addy M, Wade W (1988):** The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. *J Clin Periodontol*; 15: 415.
- Kalaga A, Addy M. Hunter B (1989)** Comparison of Chlorhexidine Delivery by Mouthwash and Spray on Plaque Accumulation. *J. Periodontol* 1989; 60(3): 127-129.
- Kornman KS (1986):** The role of supragingival plaque in the prevention and treatment of periodontal diseases; a review of current concepts. *J Periodont Res*; 21 (supp 16): 5-22.
- Kramer A, Hoppe H, Krull B, Pitten FA, Roseanu S (1998).** (Antiseptic efficacy and acceptance of Octenisept computed with common antiseptic mouthwash). (German) *Zentralblatt fur Hygiene and Umweltmedizin*; 200(5-6): 443-56.
- Leenstra TS, Van Saene JJ, Van Saene HK, Martin MV (1996):** Oral endotoxin in health adults. *Oral surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics.* 1996; 82 (6); 637-43.
- Lerardi E, Amoruso A, La No He T, Francavilla R, Marrazza E. Monno RA, Francavilla A (1980);** Halitosis and *Helicobacter - pylori*: A possible relationship. *Digestive Disease and Sciences*; 43 (12): 2733-7.
- Lockhart PB (1996):** An analysis of bacteremia during dental extractions. A double-blind, placebo-controlled study of Chlorhexidine (Review) *Arch Int Med*; 156 (5): 513 -30.
- Okuda K, Adachi M, Iijima K (1998):** The efficacy of antimicrobial mouthrinses in oral healthcare. (Review). 1998; 39 (1): 7- 14.
- Pitts G, Brogdon C, Hu L, Masurat J, Pianotti R, Schumann P (1983).** Mechanism of action of an antiseptic anti-odor mouthwash. *J Dent Res* 1983; 62:738-742.
- Sardella A, Carrassi A, Caputo R, Monti M, Gotte P (1998).** Gingival lesions in-patients with dermochoondrocorneal dystrophy (Francois Syndrome). A Case report (Review) *J. Clin Periodontol*; 25(12) : 1047-9

Received: December, 2002

Accepted in final form: May 2003