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Effect of essential oils containing and alcohol-free chlorhexidine mouthrinses on cariogenic micro-organisms in human saliva

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Abstract

Objective. The aim of this study was to evaluate the effect on mutans streptococci and lactobacilli in saliva of mouthrinsing with essential oils and an alcohol-free chlorhexidine. ***Materials and method.*** Twenty healthy volunteers (mean age 59 years) participated in the double-blind randomized cross-over study. Three mouthrinses were used in 16 days rinsing periods in addition to their regular mechanical oral hygiene: a solution with essential oils (EO; Listerine), a solution with alcohol-free chlorhexidine (CHX; Paroex) and water (negative control). The mouthrinse periods were separated by 3-month washout periods. At days 0 (baseline) and 17 (end) of each mouthrinse period, paraffin stimulated whole saliva was collected in order to analyse CFU/ml saliva of mutans streptococci and lactobacilli. ***Results.*** Only the CHX rinse showed a significant difference for CFU mutans streptococci between baseline and end ($p = 0.004$). The CFU mutans streptococci at the end of the rinse periods showed statistically significant differences between CHX vs EO ($p = 0.039$) and CHX vs water ($p = 0.022$). The difference in CFU lactobacilli between baseline and end was significant for CHX ($p = 0.031$), but not for the other rinses. No statistically significant differences for lactobacilli were found at the end of the rinse periods between the mouthrinses. ***Conclusion.*** A significant reduction in amount of cariogenic bacteria in saliva was observed after 16 days of alcohol-free chlorhexidine mouthrinse but not after the essential oils rinse. The high number of participant's not changing to a bacterial class with a reduced number of micro-organisms showed that both rinses had little clinical significance as a caries preventing treatment method, which can decrease the number of CFU cariogenic micro-organisms.

Key Words: *bacteria, caries, chlorhexidine, clinical, essential oils, mouthrinse, saliva*

Introduction

Mutans streptococci and lactobacilli are micro-organisms closely associated with the development and progression of dental caries. Caries can be considered as a behavioural disease, which can be prevented simply by good oral hygiene and restricting the frequency of fermentable carbohydrate intake. Modern dentistry emphasizes the importance of dental plaque control to improve oral health. Mechanical removal of plaque requires manual dexterity and time and few people can consistently maintain a plaque-free status. Chemical plaque control is based on the potential deficiencies of mechanical cleaning and may be beneficial as an adjunct to fluoride and sugar restriction [1]. Several clinical studies have studied the effectiveness of chlorhexidine and essential oils, the most widely used antimicrobial mouthrinses, in treating supragingival plaque and gingivitis [2,3]. Cariogenic activity may also be influenced by reducing the number of cariogenic micro-organism in dental plaque [1,4]. Chlorhexidine, the most widely-tested and effective anti-plaque agent, reduces the oral level of mutans streptococci by their ability to bind to the bacterial cell membrane surfaces and changes in membrane permeability [4,5]. Its use is restricted to short-time use because of some reversible local side-effects. The evidence of the effect of essential oils on reduction of cariogenic micro-organisms is very limited [6,7]. Various oral mouthwashes contain denatured alcohol as a vehicle to dissolve and deliver antiseptic ingredients in relative high concentrations (12–27%). There is a concern that alcohol can cause oral dryness, oral pain, epithelial desquamation and increased risk of oropharyngeal cancer [8,9]. McCullough and Farah [10]

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reported in a recent review that there was sufficient evidence that alcohol is involved in the development of oral cancer. Werner and Seymour [11] reviewed this paper and an updated review of La Vecchia (2009) about mouthwash and oral cancer risk [12]. They concluded that there is evidence showing the existence of this association, but these are still weak and inconclusive and a robust randomized clinical trial would be necessary to verify the hypothesis. The presence of alcohol has been suggested to be contra-indicated for patient groups like subjects with muco-sitis, patients with sensitive oral tissues by disease or treatment or subjects sensitive for alcohol [6]. Not until recently, an alcohol-free chlorhexidine mou-thrinse was introduced on the Scandinavian market with limited clinical evidence of its effectiveness [6,13,14].

Recently, Wikén Albertsson et al. [15] showed by intra-oral pH measurements with a microtouch electrode a decreased plaque acidogenicity in subjects using alcohol-free chlorhexidine or essential oils con-taining mouthrinses after a sucrose challange. The decrease in acidogenicity was suggested to be attributed to reduction in plaque amount, changed plaque com-position, plaque viability, changes in plaque metabolism or to a suppression of cariogenic micro-organisms. The efficacy of different modes of alcohol-containing chlor-hexidine treatments on mutans streptococci in plaque and saliva have been reviewed by Emilsson [1]. The efficacy of alcohol-free chlorhexidine and essential oils mouthrinse to suppress the cariogenic micro-organisms mutans streptococci and lactobacilli is not known. Lindqvist et al. [16] found a positive correlation between the salivary concentration of mutans streptococci and their presence in the dentition. The aim of this study was to evaluate, in an intra-individual comparison, the reduction of mutans streptococci and lactobacilli in saliva after rinsing with essential oils containing mou-thrinse and an alcohol-free chlorhexidine. The hypoth-esis tested was that the amount of mutans streptococci and lactobacilli in saliva would be similar after rinsing with the antimicrobial solutions.

Materials and methods

Test subjects

Twenty healthy volunteers (12 men and eight women) with a mean age of 58.9 years (range 42–90 years), recruited at the Dental School, University of Umeå, Sweden, participated in the study. The number of participants was based on earlier similar clinical eva-luations in our research group [17]. All, except two, subjects had a history of high caries frequency. All teeth were without obvious active caries. None of the subjects had a history of antibiotic therapy during the previous 6 months. Oral and written information was given to the subjects at the first visit. All subjects gave their informed consent prior to the start. The study was approved by the Regional Research Ethics Committee at the Medical Faculty, Umeå University, Sweden (Dnr 08-072M).

Study design

The study was designed as a double blind randomized intra-individual comparison of three mouthrinses: (1) a solution containing essential oils (Listerine fluoride, McNeil, Stockholm, Sweden), 20 ml, (2) a solution with 0.12% alcohol-free chlorhexidine (Paroex; Sunstar Butler, Mölndal, Sweden), 10 ml and (3) water (negative control), 10 ml. Each subject partici-pated in each rinse group in a randomized order. The randomization was performed at the start of the study by drawing of lots. Each product was used during a test period lasting for 16 days in addition to their regular mechanical oral hygiene procedures and nor-mal dietary practise (Figure 1). The subjects refrained from brushing for 3 days before each mouthrinse started and the last 3 days of each rinsing period for planned plaque sampling and analysis. The different mouthrinse periods, which were carried out in randomized order, were separated by 3-month washout periods. The unsupervised rinsings were initiated after a baseline oral examination and saliva sampling (Day 0). The amount and application of the products were used according to the manufacturer's recommendations. The subjects used the mou-thrinses once on Day 0 and twice a day during Days 1–16. In order to assure compliance, they main-tained a diary to document the rinses as well as the performance of the daily mechanical oral hygiene procedures.

Sampling and analyses

The saliva sampling sessions were performed at the dental clinic, School of Dentistry, University of Umeå. Each subjects attended six sessions, if possible at 10 am; at both baseline (Day 0) and at the end of the three mouthrinse periods (Day 17). The subjects were not allowed to eat, drink or use tobacco for 2 h prior to the saliva sampling. After the assessments, each subject received a professional cleaning by one of the authors [18]. At the six sessions of days 0 and 17 of each mouthrinse period, 5 ml paraffin stimulated whole saliva was collected for microbiological and biochemical saliva analyses. For bacterial analyses, performed directly after sampling, 1 ml saliva was taken and added to 4 ml salt buffer solution. To determine the number of mutans streptococci and lactobacilli, aliquots of the samples were spread on mitis salivarius agar (Difco, Becton, Dickinson and Company, Sparks, MD) supplemented with bacitracin [18] and on Rogosa selective lactobacilli agar (Merck, Darmstadt, Germany), respectively. To determine the total

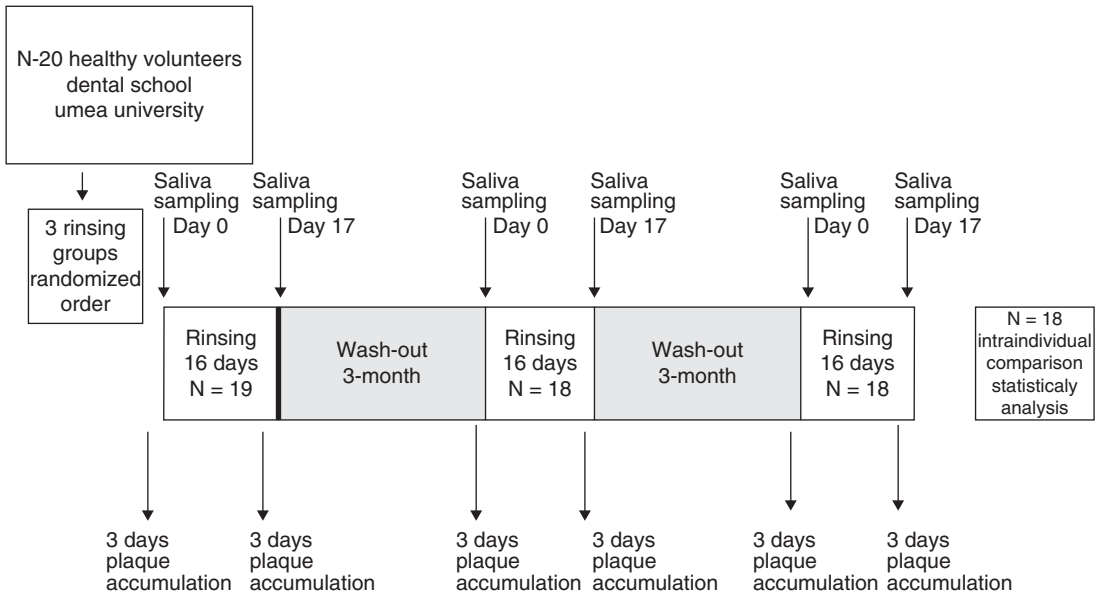

Figure 1. Flow-diagram of the study design.

number of bacteria in the sample, aliquots were spread on blood agar plates. After 2 days incubation in 5% CO_2 and 95% air at 37°C, all colony forming units (CFU) on the blood agar plates were counted.

Statistical analysis

The data were processed in SPSS (Statistical Package for the Social Sciences, version 15.0, Chicago, IL). Frequency distributions of colony forming unit (CFU) mutans streptococci and lactobacilli were performed. CFU values of mutans streptococci and lactobacilli at baseline were, due to the high variance of cariogenic micro-organisms between individuals, classified into five classes with 10× power differences before statistical analysis Classification of CFU mutans streptococci: Class 1: 0–$<10^5$, Class 2: 10^5–$<10^6$, Class 3: 10^6–$<10^7$, Class 4: 10^7–$<10^8$, Class 5: 10^8+. Classification of CFU lactobacilli: Class 1: 0–$<10^4$, Class 2: 10^4–$<10^5$, Class 3: 10^5–$<10^6$, Class 4: 10^6–10^7, Class 5: 10^7+.

CFU obtained at each assessment and bacterial type were transformed to $\log_{10}$ and preformed in order to normalize their distribution. Intra-individual comparisons of the observed micro-organisms in saliva within each mouthrinse group, before vs after mouthrinsing, and between the three mouthrinses both before and after the rinsing period, using $\log_{10}$-transformed CFU counts, were made using Wilcoxon's signed rank test. p-values < 0.05 were considered statistically significant.

Results

Eighteen of the original 20 subjects successfully completed all three series of rinses. One participant did attend only two sessions and a second discontinued the rinses. No adverse effects were reported by any of the subjects during or after the rinses. Large inter-individual variations were observed for the levels of mutans streptococci and lactobacilli. The median (SEM) values, CFU/ml saliva values of the counted micro-organisms at baseline and at the end of each mouthrinse are shown in Table I. The relative distributions (%) of CFU mutans streptococci and lactobacilli in the five classes at both baseline and at the end of the three rinsing periods are shown in Table II. Change of bacterial classification of the subjects, for mutans streptococci and lactobacilli, at the end of the rinsing periods compared with their baseline classification is shown in Table III.

The alcohol-free CHX showed significantly decreased amounts of CFU mutans streptococci/ml saliva ($p = 0.004$) and lactobacilli ($p = 0.031$) at the end of the rinse period. The changes between baseline and end

Table I. Absolute frequencies of CFU mutans streptococci and lactobacilli per ml saliva at start (baseline = B; day 0) and end (E; day 17) of the three mouthrinse periods (median, SEM).

	Mutans streptococci		Lactobacilli	
Rinse	Median	SEM	Median	SEM
CHX B	1.2×10^6	1.2×10^6	6.4×10^4	4.3×10^5
CHX E	5.3×10^3	3.5×10^7	2.1×10^4	4.0×10^5
EO B	0.6×10^6	2.8×10^6	6.0×10^4	8.2×10^5
EO E	1.0×10^6	1.0×10^6	6.3×10^4	4.1×10^5
Water B	1.1×10^6	2.2×10^6	6.3×10^6	7.2×10^5
Water E	1.0×10^6	2.4×10^6	7.3×10^5	6.9×10^5

CHX, Chlorhexidine (Paroex); EO, essential oils (Listerine).

Table II. Relative distributions (%) of CFU mutans streptococci and lactobacilli within five bacterial classes* at baseline and the end of the respective mouthrinse periods.

Classs	CHX B	CHX E	EO B	EO E	Water B	Water E
Mutans streptococci						
1	22.2	66.7	38.9	22.2	27.8	22.2
2	22.2	11.1	16.7	22.2	22.2	27.8
3	38.9	5.6	22.2	38.9	33.3	22.2
4	11.1	11.1	5.6	5.6	5.6	16.7
5	5.6	5.6	16.7	11.1	11.1	11.1
1–5	100	100	100	100	100	100
Lactobacilli						
1	16.7	33.3	33.3	22.2	22.2	22.2
2	38.9	38.9	22.2	33.3	38.9	33.3
3	11.1	5.6	11.1	22.2	16.7	22.2
4	33.3	22.2	22.2	22.2	16.7	16.7
5	0	0	11.1	0	5.6	5.6
1–5	100	100	100	100	100	100

*Classification of CFU mutans streptococci; Class 1: 0–$<10^5$, Class 2: 10^5–$<10^6$, Class 3: 10^6–$<10^7$, Class 4: 10^7–$<10^8$, Class 5: 10^8+. Classification of CFU lactobacilli; Class 1: 0–$<10^4$, Class 2: 10^4–$<10^5$, Class 3: 10^5–$<10^6$, Class 4: 10^6–10^7, Class 5: 10^7+.
CHX, Chlorhexidine (Paroex); EO, essential oils (Listerine); B, baseline (day 0); E, end (day 17).

of the EO and water rinses were not statistically significant, neither for mutans streptococci ($p = 0.453$ and $p = 0.344$, respectively) nor for lactobacilli ($p = 1.0$ and $p = 0.344$, respectively). Change of mutans streptococci and Lactobacilli counts during the 17 days mouthrinsing periods for the three rinses; CHX, EO and water, given as $\log_{10}$ CFU mutans streptococci/ml saliva for each of the participating subjects, are shown in Figures 2 and 3.

No significant differences in CFU counts for mutans streptococci or lactobacilli were observed at baseline between the three rinsing groups. At the end of the rinse periods, statistically significant differences were observed for mutans streptococci between CHX and EO ($p = 0.039$), and between CHX and water ($p = 0.022$). No statistical difference was seen for EO vs water ($p = 1.0$). No statistically significant differences between the three rinsing groups were observed at the end of the rinse periods for CFU lactobacilli.

Table III. Differences in bacterial classification* of the individual subjects at the end of the rinsing periods compared with their baseline classification.

	Rinsing group	−2	−1	0	+1	+2
Mutans	Water	0	7	8	2	1
Streptoccocci	Essential oils	2	3	11	0	2
	Chlorhexidine	0	0	9	4	5
Lactobacilli	Water	0	5	10	2	1
	Essential oils	1	4	8	2	3
	Chlorhexidine	0	0	12	4	2

*Classification of CFU mutans streptococci; Class 1: 0–$<10^5$, Class 2: 10^5–$<10^6$, Class 3: 10^6–$<10^7$, Class 4: 10^7–$<10^8$, Class 5: 10^8+. Classification of CFU lactobacilli; Class 1: 0–$<10^4$, Class 2: 10^4–$<10^5$, Class 3: 10^5–$<10^6$, Class 4: 10^6–10^7, Class 5: 10^7+.
Number of subjects with higher CFU counts classes at the end of the rinsing period: −2 = 2 classes, −1 = one class difference. The same class = 0. Lower CFU counts class at the end: +1 = one class, +2 = 2 classes.

Discussion

Reduction in levels of cariogenic bacteria in plaque is one of the main approaches to prevent caries. The ability of antimicrobial mouthrinses to significantly reduce levels of cariogenic micro-organism would therefore provide an additional rationale for their use as part of a daily oral hygiene regimen [17]. Alcohol containing CHX has been studied extensively during the last 35 years and is still the most potent chemotherapeutic agent against mutans streptococci and used as a caries preventive agent [17,19]. Consequently, CHX is often used as a positive control for assessment of other agents with anti-cariogenic potential. The main finding in the present study was that a rinsing period of 16 days with the 0.12% alcohol-free CHX significantly reduced the CFU mutans streptococci and lactobacilli in saliva, while no differences were observed after the essential oils and water rinse. The hypothesis was, therefore, rejected.

The intra-individual cross-over design avoids highly variable individual confounding influences like mechanical cleaning, diet and salivary quality and quantity, which may differ dramatically between subjects and may mask the effect of the tested chemical agents [20]. It takes considerabe power to detect statistical differences between agents [21]. The labour

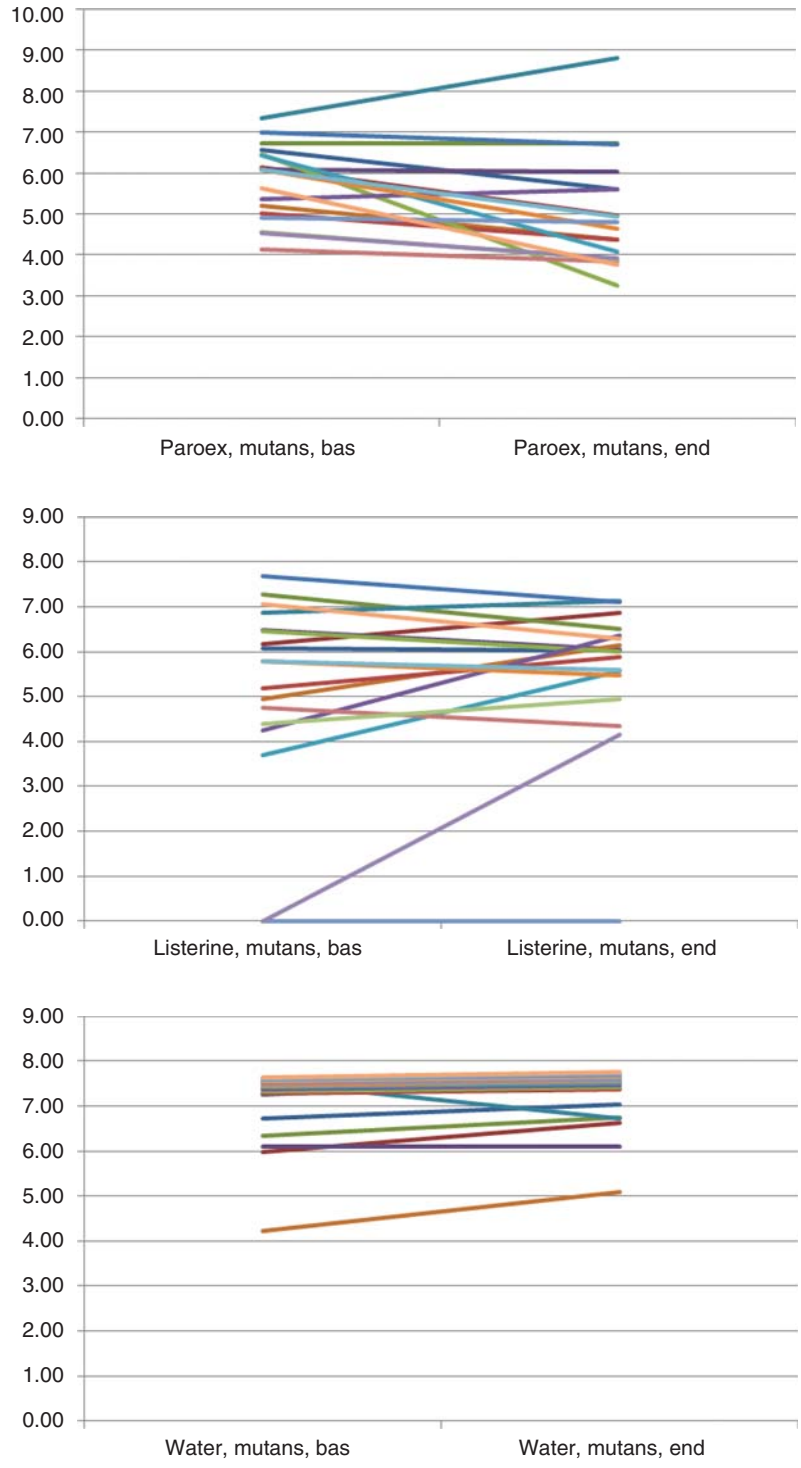

Figure 2. Change of mutans streptococci counts during the 17 days mouthrinsing periods for the three rinses; Chlorhexidine (Paroex), essential oils (Listerine) and water. Shown as $\log_{10}$ CFU mutans streptococci/ml saliva counts at day 0 and day 17. Each line represent one of the participating subjects, $n = 18$. mutans = mutans streptococci, Bas = baseline (day 0), End = day 17.

intensity and long-term character of these studies, determined by time of rinsing and length of washout period, will, however, restrict the number of involved agents. The large variability in the microbial counts observed in the present study may impair the ability to demonstrate effect on micro-organisms. To stabilize the high variability in the counts, data were subjected to logarithmic transformations.

Carry-over of the effect of a mouthrinse agent of one treatment period to the next is a potential problem for cross-over studies. The long 3 months washout period in this study was chosen based on the

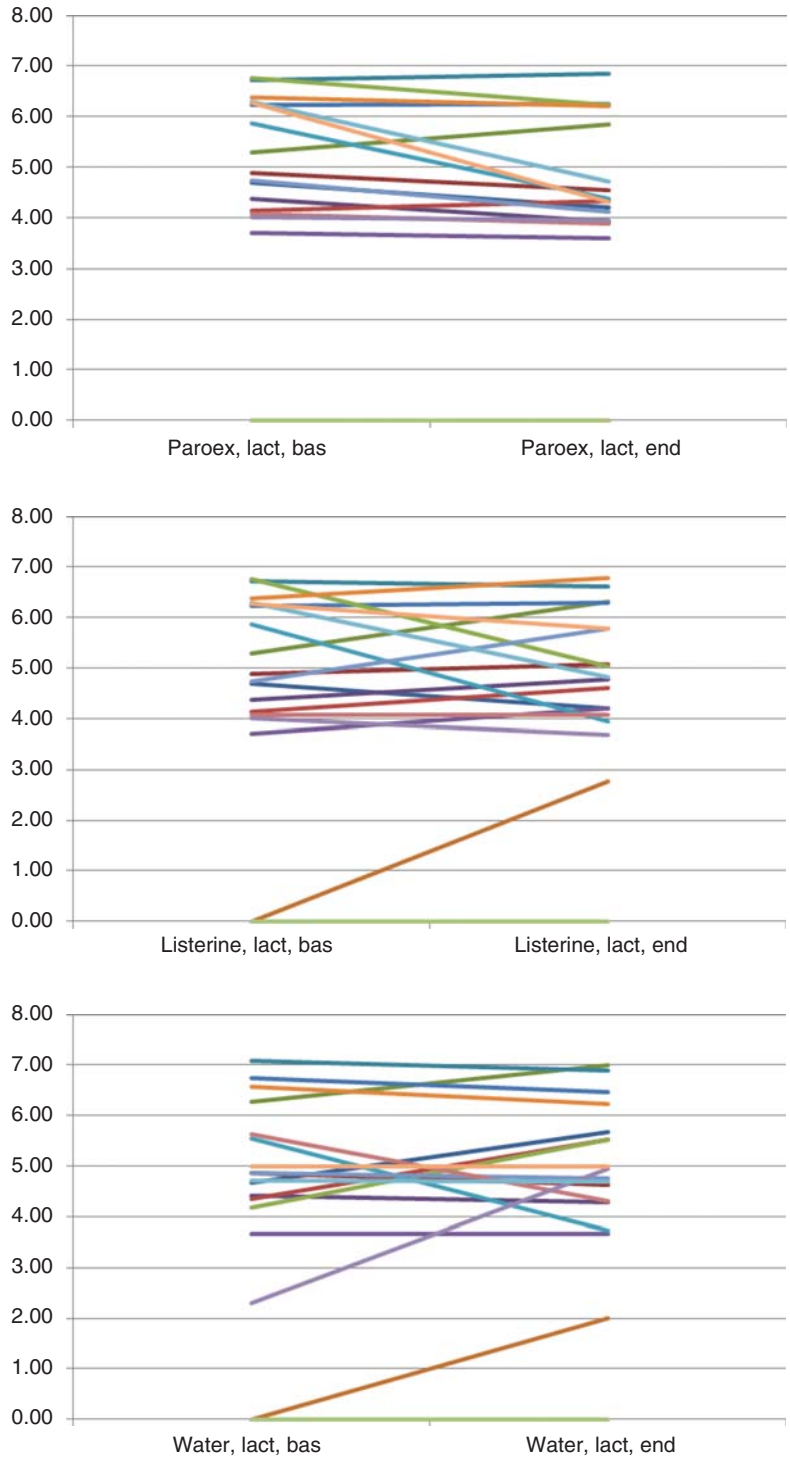

Figure 3. Change of lactobacilli counts during the 17 days mouthrinsing periods for the three rinses; Chlorhexidine (Paroex), essential oils (Listerine) and water. Shown as $\log_{10}$ CFU lactobacilli/ml saliva counts at day 0 and day 17. Each line represent one of the participating subjects, $n = 18$. lact = lactobacilli, Bas = baseline (day 0), End = day 17.

bacterial recolonization time to baseline levels after intensive treatment with CHX gel studies [22,23]. CHX gel application exerts a more efficient long-lasting suppression of mutans streptococci than CHX rinse and a shorter washout period could, therefore, have been chosen [17,24]. Rate of recovery will be dependent on the rate of suppression, which will be influenced by the concentration and time of use of the antimicrobial agent [20,25,26]. The antibacterial effects of single CHX mouthrinses showed a decrease of S. mutans during the first hours after the mouthrinse, with a subsequent recovery at 7 h after the rinse [13,14]. Persson et al. [27] observed, after a 6 week rinsing with 0.12%, a still significantly lower

S. mutans scores after 12 weeks. Increasing the rinsing period implies longer recovery periods [28]. Several earlier studies used very short wash-out periods, which include a risk that the bacterial flora still is influenced by the earlier antimicrobial treatment and has not be re-established [7,29,30]. Emilson et al. [24] found that the re-appearance of mutans streptococci was proportionally slower in saliva than on the teeth surfaces after treatment with 1% CHX gel, indicating that the salivary levels were under-estimated in the dentition [24]. Most studies using a 0.12% CHX could not show long-term microbial reduction [26,31]. In a systematic review, Ribeiro et al. [4] concluded that rinsing with a CHX mouthwash solution had no long-term effect on salivary mutans streptococci. In the present study, the effect of rinsing was determined directly after the end of the rinsing period, which is the most common way to evaluate [6,14,20,28,29]. Due to the randomized, cross-over design of the present study it was not possible to observe the long-term effect of the rinses.

Mutans streptococci levels in plaque and saliva have been suggested to be an aid in the diagnosis of caries activity [32]. and Lindqvist et al. [16] showed earlier that CFU in stimulated saliva reflects the detection frequencies of the organism on tooth surfaces. To be able to compare the effects of the different treatments on CFU plaque counts, plaque sampling has to be standardized according to weight or volume to make intra- and inter-individual comparisons possible. Due to large differences in plaque liquid contents, saliva sampling is easier to perform and standardize. Saliva sampling after chewing on paraffin was chosen in this study. To remove the micro-organisms adhered to the tooth surfaces, chewing on paraffin was preferred in several studies [16,27,28,33,34]. It is, therefore, surprising that several recent studies are based on sampling with resting saliva or rinsing with water or saline, methods which will be far less effective to remove plaque micro-organisms from the tooth surfaces into saliva and without evidence [7,13,14,26,30,31]. Large variations are seen in CHX mouthwash studies concerning quantity (4–15 ml), rinsing occurrence (4-times daily–once weekly), rinsing time (30–120 s), rinsing periods (single rinse–6 weeks) [4,6,13,14,30,31]. The heterogeneity of the methods applied makes the comparison of results between the different studies difficult. Many earlier studies observed the antimicrobial effect of alcohol containing CHX mouthrinses in reducing mutans streptococci [6,20,25,35]. However, other studies could not reproduce the effect [26,33,36,37]. Dahlén [33] found no significant reduction for S. mutans in stimulated saliva when rinsing twice a day with 0.2% CHX for 1 week. A significant reduction was found after 4-rinses a day for 1 week. Zanela et al. [37] found that CHX solutions do not reduce the levels of mutans streptococci unless they are initially high, as earlier reported by Zickert et al. [19] and Emilson et al. [24], in contrast with the conditions presented in this study.

In most studies either a 0.12% or a 0.2% CHX mouthwash were used. Clark and Guest [20] showed that the minimum effective concentration of alcohol containing CHX mouthrinses is 0.12%. Lower concentrations failed to significantly reduce the counts of cariogenic bacteria in saliva [20]. In their study a 0.12% alcohol containing CHX mouthwash was used while the participants still used their own oral hygiene procedures. Few studies evaluated the effect of alcohol-free CHX, but all confirmed the present study's effect on mutans streptococci [6,13,14]. Eldridge et al. [6] showed in a non-cross-over design that both an alcohol containing and an experimental alcohol-free CHX were effective in reducing saliva levels of mutans streptococci as measured with an S.mutans strip (score 0), despite the absence of mechanical oral hygiene. The reduction of mutans streptococci after a single rinse, with the same CHX as in the present study, was confirmed by Tomas et al. [13], which persisted up to 5 h after the mouthrinse. Two other alcohol CHX mouthrinses (0.2% and 0.12%) produced a significant antibacterial effect at 7 h [13]. Cousido et al. [14] observed a total recovery of bacterial vitality in unstimulated saliva after a single mouthwash with the same 0.12% alcohol-free CHX after 7 h. An increased rinsing period with a 0.12% CHX twice a day reduced significantly S. mutans in stimulated saliva and persisted in samples taken at 24 h [28]. The present study confirmed that the alcohol-free 0.12% CHX mouthrinse like the alcohol containing ones reduced significantly mutans streptocooci in saliva. On the individual level, 50% of the participants showed reduced levels. However, the other 50% remained CFU counts in the same bacterial class. The potential of the rinse as a general cariogenic preventing treatment has therefore to be questioned.

Pan et al. [35] demonstrated with a fluorescent stain technique that rinsing with EO has marketed bactericidal effects against bacteria within plaque *in situ*. Haffajee et al. [38] showed *in vitro* that herbal mouthrinses and 0.12% CHX showed significantly lower oral bacteria, including S. mutans, MIC values than EO. Filoche et al. [39] indicated a potential role of essential oils in the development of novel anti-caries treatments. Limited studies evaluated the effect of EO on cariogenic micro-organisms. Fine et al. [7] reported that rinsing with an essential oil mouthrinse resulted in significant reductions in both S. mutans and total streptococci in interproximal plaque and saliva after 2 days daily rinse during 12 days. However, their plaque sampling method was not standardized concerning volume or weight and conclusions are impossible to make. The reported antimicrobial

effect of the agent on CFU mutans streptococci is probably caused by the total plaque-reducing effect of the agent after the rinse. Plaque sampling to assess the effect of chemical plaque control on cariogenic micro-organisms request standardization of the method by either volume or weight. In unstimulated saliva samples, Fine et al. [7] reported a significant 39.2% reduction in total recoverable S. mutans counts. The decrease in the proportion of S. mutans to total streptococci in saliva was, however, not significant. There is to our knowledge no study that showed the relationship between the amount of mutans streptococci in plaque vs those observed in non-stimulated saliva. The value of the reported reduction of the CFU has, therefore, to be questioned. The effect of an EO rinse on interproximal plaque bacteria as assessed by optical density measurement showed mean $\log_{10}$ CFU/ml values not statistically significant from the negative water control rinse [29]. This internal study by the manufacturer, like the studies by Fine et al. [7,40] used a non-weight controlled plaque sampling with paper points. Eldridge et al. [6] compared in three student groups the effectiveness of EO with an alcohol containing and an alcohol-free CHX on mutans streptococci in saliva. Both CHX groups showed a significant difference with the EO. Relative microbial growth for both CHX products decreased to 0 after 21 days (Dentocult scores) compared to 36% in the EO group. Eldridge et al. [6] observed a large colony counts variance in the EO group which was confirmed in the present study. As shown in Table III, the EO rinse showed no reliable way to reduce mutans streptococci. Eleven of the 18 participants showed no change, while five showed an increased CFU in mutans streptococci, comparable to the water rinse group.

Lactobacillus species are less susceptible to CHX and will survive levels bactericidal to mutans streptococci [6,19,22]. Very few clinical studies evaluated the effect of alcohol containing CHX mouthrinses and no study of alcohol-free CHX on lactobacilli counts *in vivo*. Persson et al. [27] studied the effect of daily and weekly rinsing with an alcohol containing 0.12% CHX during 6 weeks on salivary lactobacilli in a geriatric population. A significant decline of lactobacilli counts for the group as a whole was observed, but 62% of the weekly group still had high counts of lactobacillus, placing them at risk. No statistically significant differences between baseline and 6 weeks lactobacilli counts were observed for the daily rinse group. In the present study, the alcohol-free CHX showed a significant reduction of lactobacilli counts. However, 12 of 18 participants did not show a change in bacterial class (Table III). The water and EO rinses showed no significant changes in CFU lactobacillus. The majority of the participants showed no effect of the EO rinse on lactobacilli.

The observed proximal plaque acidogenicity reduction after the CHX mouthrinse by Wikén Albertsson et al. [15] observed with the micotouch method may for a large part be explained by the reduction in mutans streptococci and lactobacilli in the present study. For the EO rinse, the reduced acidinogenicity, significant difference with the water control at 5 min after the sucrose challenge, may be attributed to other factors than a decrease in CFU of the studied micro-organisms. However, it has to be observed that the present bacterial results are based on measurements of occlusal plaque micro-organisms lost during the chewing on a piece of paraffin, while the micro-touch values are based on proximal plaque measurements. EO may penetrate deeper in the non-removed proximal plaque than in the thinner occlusal plaque and influence bacteria metabolism after accumulation. On the other hand, it may also be possible that EO is more effective on other acid-producing micro-organisms, like non-mutans streptococci, than the ones studied. The earlier shown reduced acidogenicity after sucrose challenge indicate that EO has a limited caries preventive potential.

Conclusion

The alcohol-free chlorhexidine used as mouthrinse resulted in a significant reduction in mutans streptococci and lactobacilli, while the EO rinse showed no such reduction.

The high number of participant's not changing to a bacterial class with reduced micro-organisms showed that both rinses had no clinical significance as a caries-preventing treatment method.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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