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# Impact of mouthrinses on morning bad breath in healthy subjects

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#### Abstract

**Background:** During sleep, a proliferation of oral bacteria is responsible for the release of offending gases in morning breath even in healthy people. Thus, the aim of this study was to evaluate the bad breath-inhibiting effect of four commercially available mouthrinses (0.03% triclosan, 0.12% chlorhexidine gluconate, 0.05% cetylpyridinium chloride and essential oils) on morning breath when compared with a positive and a negative control.

**Method:** A six-step double-blind, crossover, randomised study was conducted in 12 dental students with healthy periodontium, who refrained from mechanical plaque control during a 4-day period. The subjects were instructed to rinse twice daily with the assigned product during each period. Fifteen-day washout intervals were used. Before professional plaque and tongue coating removal (baseline), the morning breath was scored through volatile sulphur compounds (VSCs) level measured by a sulphide monitor. After 4 days, VSCs and plaque index (PI) were recorded.

**Results:** Even in the absence of mechanical plaque control, there was a decrease in VSC level with the use of all mouthrinses, with the exception of an increase with the use of the negative control. The VSC formation was inhibited in descending order, by positive control (0.2% chlorhexidine), 0.12% chlorhexidine, triclosan and essential oils and cetylpyridinium chloride. Plaque formation was inhibited by chlorhexidine mouthrinses and essential oils.

**Conclusions:** These findings suggest that mouthrinses can reduce morning bad breath, and that such a reduction is not attributable only to the reduction of supragingival plaque formation.

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Breath malodour is a problem for many individuals. It usually originates within the oral cavity itself (Loesche et al. 1985, Simonson et al. 1988, Kleinberg & Westbay 1990, Delanghe et al. 1997, 1999, Söder et al. 2000) due to the degradation of proteins by specific bacteria (Tonzetich 1977, Rosenberg et al. 1991a, b) with the production of volatile sulphur compounds (VSCs).

A number of factors, both intra- and extra-oral, such as gingivitis, periodontitis, nasal inflammation, chronic sinusitis, diabetes mellitus, liver insufficiency, cirrhosis, uraemia, lung carcinoma, trimethylaminuria and post-nasal drip have been identified (Preti et al. 1995, Newman 1996, van Steenberghe 1997, Nogueira-Filho et al. 2002). Delanghe et al. (1997, 1999) examined hundreds of patients with bad breath and found that in around 87% of them, bad breath originated from oral causes, whereas only 5–8% of cases were attributed to ear, nose and throat causes.

There is ample evidence to show that VSCs in breath increase with the number, depth and bleeding tendency of periodontal pockets (Tonzetich 1973, Persson 1992, Coli & Tonzetich 1992, Yaegaki 1995, Ratcliff & Johnson 1999, Morita & Wang 2001). Although periodontal disease can be a strong factor in chronic halitosis (Söder et al. 2000), it is well documented that the tongue surface is another strong odour formation site in the mouth (Yaegaki & Sanada 1992, Miyazaki et al. 1995). As a matter of fact, a substantial proportion of healthy people complain of oral malodour (Yaegaki & Sanada 1992, Bosy et al. 1994).

The antiplaque efficacy of many chemical agents present in dentifrices and mouthrinses has been evaluated for chemical plaque control (Hull 1980, Addy 1986, Kornman 1986, Axelsson & Lindhe 1987, Ciancio 1995, Nogueira-Filho et al. 2000). Mouthrinsing, in particular, is a common oral hygiene practice dating back to ancient times (Mandel 1988).

The major concern that leads to the frequent use of mouthrinses is halitosis (Wennström 1988). According to Loesche (1999), early clinical trials of antimalodour mouthrinses have been designed with a drug or cosmetic claim (Schmidt & Tarbet 1978, Pitts et al. 1983, Yaegaki & Suetaka 1989, Rosenberg et al. 1992, Kozlovsky et al. 1996, Nachnani 1997). Therefore, recent reports have pointed out the relevance of comparative studies to verify the real efficacy of such mouthrinses (Silwood et al. 2001, van Steenberghe et al. 2001, Rösing et al. 2002).

Healthy individuals who complain of bad breath (Rosenberg 1995) have been using mouthrinses containing several masking and antimicrobial agents (Rosenberg 1992, van Steenberghe 1997). Their clinical efficacy has often been tested on morning breath (Tonzetich 1976, Hoshi & van Steenberghe 1996), rather than in real situations, for evident ethical reasons. The morning breath odour can be used as a model to investigate other offensive breath odours and it is universally accepted (van Steenberghe et al. 2001). Thus, the aim of this study was to evaluate the bad breath-inhibiting effect of four commercially available antiplaque mouthrinses on morning breath.

# Material and Methods Patient population

Twelve dental students, five females and seven males (aged 19–23 years) from the Faculty of Dentistry of Piracicaba-UNICAMP, volunteered to participate in this study. The Institutional Committee of Ethics in Clinical Research of the University of Campinas approved the study protocol. The exclusion criteria were subjects with medical disorders, undergoing antibiotic or other antimicrobial therapy, smokers, pregnant women and those who, on pre-study clinical screening, presented a probing depth of  $\geq$  3 mm with bleeding on probing and with less than 20 natural teeth.

#### Study design

This study was a randomised, doubleblind comparison of 12 volunteers divided into six crossover groups performed in six experimental periods of 4 days. Each period was followed by a 15day washout interval. A 4-day plaque regrowth experimental model was adopted (Addy et al. 1983).

#### Pre-experimental phase

A 15-day pre-experimental phase occurred, where the subjects used a dentifrice without antimicrobial agents (Sorriso<sup>®</sup>, Anakol Ind. Com. Ltda, Kolynos do Brasil, Colgate Palmolive Co, São Bernardo do Campo, SP, Brazil). The baseline data of dental plaque – plaque index (PI) (Silness & Löe 1964) and gingival – gingival index (GI) (Talbott et al. 1977) indices were recorded.

#### Test and control products

Four commercial mouthrinses were bought in the market from different dealers and repacked in white bottles to ensure blindness of the study:  $Cepacol^{\mathbb{R}} = 0.05\%$ cetylpyridinium chloride (Gessy Lever Co., Unilever Vinhedo. SP. Division. Brazil):  $Plax^{\mathbb{R}} = 0.03\%$  triclosan + 0.2% copolymer (Colgate Palmolive, Division of Kolynos do Brasil Ltda, Osasco, SP, Brazil); Listerine<sup> $\mathbb{R}$ </sup> = 0.064% thymol, 0.09% eucaliptol and 0.042% menthol (essential oils) (Procter & Gamble Laboratories, Surrey, UK); Peri $ogard^{\mathbb{R}} = 0.12\%$  chlorhexidine gluconate (CHX) (Colgate Palmolive, Division of Kolynos do Brasil Ltda, Osasco, SP, Brazil).

A positive control containing 0.2% CHX and negative hydro-alcoholic control were made by Proderma Laboratories (Piracicaba, SP, Brazil). During the pre-experimental phase and washout intervals, a placebo dentifrice was used with a new toothbrush to avoid a carryover effect.

#### Experimental phase

On day 1 of each treatment period, all normal hygiene procedures were suspended for the next 4 days, except for the use of the allocated mouthrinses, which were assigned to the volunteers according to the experimental groups. After clinical measurements of PI, GI and VSCs, the 12 volunteers received a scale and polish to remove all plaque, calculus and stain. Professional tongue cleaning was also performed in order to eliminate any remaining tongue coating. No oral hygiene instructions were given, according to the experimental protocol. The volunteers were instructed to rinse for 1 min, twice daily at 08:00 and 16:00 hours with 15 ml of the product during the 4-day period.

#### Clinical assessment for PI and GI

The clinical assessments were performed on the mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual surfaces of the experimental teeth for gingivitis (GI) and on the mesial, buccal, distal and lingual surfaces for plaque (PI). On day 5, the PI was recorded to verify supragingival plaque formation. All measurements were performed by the same examiner, who was blind to the rinse used.

#### Morning breath evaluation

At the beginning and at the end of all experimental periods, VSC concentration was recorded using a portable industrial sulphide monitor (Halimeter<sup>®</sup>, Interscan Corp., Chatsworth, CA, USA), zeroed on ambient air before each measurement using the technique established by Rosenberg et al. (1991a, b). The data were recorded before rinsing at 08:00 hours (day 1), and 12:00 hours after the last rinse (day 5). The measurement was repeated three times for each subject.

Before the morning measurements (08:00 hours) on days 1 and 5, the volunteers refrained from toothbrushing, drinking, eating, gargling and using scented cosmetic products (Rosenberg 1996, Neiders & Ramos 1999). A Continental breakfast was offered to the volunteers after the analyses according to the criterion approved by the Ethical Committee in Research.

#### Analysis of the data

According to the crossover design, the PI on day 5 was compared among treatments applying the one-way ANOVA and post hoc LSD t-tests. For morning breath changes, the Friedman and Student-Newman-Keuls tests were applied to the data, completing the paired comparisons between VSC scores on days 1 and 5. The percentage of changes was calculated by {(VSC concentration before – VSC concentration after)  $\times$ 100/VSC concentration before}. For all the analyses, a 5% significance level was undertaken and the data were analysed using the software BioEstat 2.0 (Ayres et al. 1998).

### Results Baseline PI and GI

There were no statistically significant differences for PI and GI (p = 0.6353 and 0.8535, respectively) among the 12 volunteers at the beginning of each experimental period. Also, considering the washout periods, it seems that no carry-over effect occurred between the treatments.

# Supragingival plaque formation after 4 days

The de novo plaque formation, estimated via the Silness & Löe index, was most inhibited (p = 0.0002) by 0.2% CHX (positive control) and 0.12% CHX (Periogard<sup>®</sup>). This was significantly lower (p = 0.03) than with essential oils (Listerine<sup>®</sup>), which again was significantly (p = 0.0013) more effective than the other mouthrinses (Plax<sup>®</sup> and Cepacol<sup>®</sup>) that did not differ from the negative control (p = 0.4913 and 0.2220, respectively). These results are shown in Fig. 1.

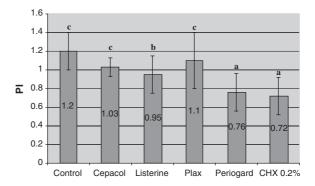
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#### Intra-group analysis

Although all volunteers refrained from any mechanical plaque control during each experimental period, some mouthrinses affected morning bad breath. The statistical analysis revealed that both 0.2 and 0.12% CHX mouthrinses presented a significant reduction (p =0.01 and 0.001, respectively) in VSC formation (Table 1). The Friedman test was not able to demonstrate significant reductions in VSC formation with the use of essential oils, triclosan and cetylpyridinium chloride mouthrinses (p = 0.30, 0.13 and 0.07, respectively).However, an increase of VSCs was observed with the use of the negative control (p = 0.05).

#### Inter-group analysis

Before the treatments, there were no statistically significant differences (p > 0.05) for VSC scores among the 12 volunteers at the beginning of each experimental period. The comparison among treatments after 4 days revealed in descending order that VSC was most inhibited by 0.2% CHX mouthrinse (p = 0.0001) followed by 0.12% CHX (p = 0.0001). This was significantly



*Fig. 1.* Means of plaque index (PI) according to the treatments. Means followed by distinct letters differ statistically (p < 0.05). Bars show SD.

*Table 1.* VSC concentration (ppb) before and after the treatments and percentage of changes (% VSC) before and after for each treatment (mean  $\pm$  SD; n = 12)

Treatments	VSC		% C VSC
	before	after	
control	$173 \pm 145^{\mathrm{aA}}$	$222 \pm 140^{\mathrm{eB}}$	20.8 <sup>c</sup>
cepacol	$169 \pm 122^{\mathrm{aA}}$	$98\pm 61^{ m dA}$	- 13.8 <sup>c</sup>
listerine	$120\pm81^{\mathrm{aA}}$	$80\pm80^{\rm cA}$	$-24.3^{b}$
plax	$150\pm118^{\mathrm{aA}}$	$81\pm86^{\mathrm{cA}}$	$-29.4^{b}$
periogard	$163\pm87^{\mathrm{aA}}$	$45\pm56^{ m bB}$	$-62.8^{a}$
chlorhexidine 0.2%	$154\pm144^{aA}$	$32\pm13^{aB}$	$-69.6^{a}$

Means followed by distinct lower letters in columns differ statistically (p < 0.05). Means followed by distinct capital letters in lines differ statistically (p < 0.05).

VSC: volatile sulphur compounds; % C VSC: % of change in VSC.

lower than essential oils and triclosan (p = 0.0001 and 0.0003, respectively), which again were significantly (p = 0.0009) more effective than the cetylpyridinium chloride, when related to negative control (Table 1). Using the concentration before the treatment as a co-variant, the percentage of changes on VSC concentration was greater by 0.2% and 0.12% CHX mouthrinses, followed by essential oils and triclosan. Cetylpyridinium chloride mouthrinses did not differ from the negative control (Table 1).

#### Discussion

The results of this investigation demonstrated the beneficial impact of mouthrinses on morning breath even in the absence of mechanical plaque control, with the exception of the negative control mouthrinse. Previous studies have shown the superiority of chlorhexidine mouthrinses on the inhibition of VSC formation (Rosenberg et al. 1992, Kozlovsky 1996, van Steenberghe et al. 2001), which is in agreement with our results. Nevertheless, the use of commercial mouthrinses containing essential oils (Listerine<sup>®</sup>), triclosan 0.03%(Plax<sup>®</sup>) and cetylpyridinium chloride (Cepacol<sup>®</sup>) did not prove to be more effective than the chlorhexidine ones in reducing VSCs.

The data of day 1 revealed high VSC scores before treatments and its reduction or increase after experimental phases (day 5), as shown in Table 1. These results demonstrate that the plaque regrowth model is effective to verify the influence of mouthrinses on VSC formation. In addition, the elevated VSC level at baseline could be explained because the volunteers were always recorded in the morning without toothbrushing and breakfast, indicating the physiologic morning bad breath related with each individual (van Steenberghe 1997).

The PI data from this study, where the model of 4-day plaque regrowth was adopted (Addy et al. 1983), pointed out the efficacy of some antiplaque agents tested against the negative control as described by in vitro studies and clinical trials (Abello et al. 1990, Jenkins et al. 1991, Moran et al. 1994, Binney et al. 1995, Riep et al. 1999, Shapiro et al. 2002). Although the group size of 12 seems to be relatively small, this investigation had sufficient power to differentiate between the chlorhexidine and other antiseptic mouthrinses. Also, the parametric statistical analyses adopted in this study were sensitive enough to verify the differences in PI among the treatments as previously reported (Jenkins et al. 1991, Moran et al. 1992, 1994, Wáler 1994).

Despite some controversy (Bosy & Geller 1996), the presence of supragingival plaque and calculus has often been associated with oral malodour (Rosenberg et al. 1991a, b, Kozlovsky et al. 1994, Söder et al. 2000, Nogueira-Filho et al. 2002). In the present investigation, the plaque formation was not always directly associated with the VSC measurements, once the triclosan and cetylpyridinium chloride mouthrinses were more effective in reducing bad breath than in supragingival plaque accumulation. Therefore, it could be postulated that the superior reducing effect of these specific mouthrinses on bad breath may be related primarily to their efficacy in reducing the load of VSC-related microorganisms and oral debris in the whole mouth niches rather than only in supragingival plaque reduction.

It was not surprising that CHX mouthrinses presented the best results in reducing morning bad breath, since this drug is of proven efficacy in the treatment of oral malodour representing a gold standard (Rosenberg et al. 1992, De Boever & Loesche 1995, Kozlovsky et al. 1996, van Steenberghe et al. 2001). Our findings complement those previously published studies in which a chlorhexidine mouthrinse was used to demonstrate that reducing VSC level (on whole mouth) and the oral microbiota (in oral niches like tongue, teeth, mucous surfaces and saliva) would reduce bad breath. On the other hand, any mouthrinse that claimed to be of a similar effectiveness as chlorhexidine. based on an antibacterial mode of action, would be inviting classification as a drug (Loesche 1999). In our results, there were no statistical differences between days 1 and 5 for VSC formation with the use of essential oils, triclosan and cetylpyridinium chloride mouthrinses.

However, the inter-group analysis showed beneficial effects of these products in morning breath when compared with the control mouthrinse. The essential oil mouthrinse (Listerine<sup>®</sup>) was able to reduce the offensive gases present in morning bad breath via a sulphide monitor, agreeing with a previous short-term study (Pitts et al. 1983), in which the results indicated a reduction of the organoleptic scores by essential oils, and caused a sustained reduction in the plaque odourigenic bacteria, unlike the placebo. The argument was made that the anti-VSC effect of Listerine<sup>®</sup> was the result of its antimicrobial effects, and this conclusion became the basis for the premise that anti-VSC agents would succeed if they had an antimicrobial component.

Triclosan (Plax<sup>®</sup>) showed a beneficial reduction in VSCs concentration, corroborating with previous reports (Niles et al. 1999, Sharma et al. 1999, Nogueira-Filho et al. 2002) that demonstrated positive results of triclosancontaining dentifrices as an anti-VSC agent, both by means of gas chromatography, organoleptically and by a sulphide monitor. Although a recent report (Rösing et al. 2002) has pointed out the questionable clinical effects of triclosan mouthrinses in reducing VSC, the experimental model used does not allow further comparisons. Our study also demonstrated that the cetylpyridinium chloride mouthrinse (Cepacol<sup>®</sup>) presented the lowest impact in reducing VSCs of morning breath when compared with the other products. This fact could be supported by the observation that this quaternary ammonium compound agent is not substantive enough to promote an essential antibacterial activity (Bonesvoll & Gjermo 1978, Addy & Wade 1995, van Steenberghe et al. 2001).

According to Dever (1979), much of the evidence of the efficacy of mouthrinses in reducing bad breath was anecdotal. Some experimental mouthrinses containing metal ion salts have proved to be effective in reducing VSC levels in short periods of time (Wáler 1997a, b, Young et al. 2001, Rösing et al. 2002). For the present study, zinc mouthrinses were not included because of a lack of commercial products in the Brazilian market. Therefore, concern should arise about mouthrinses containing zinc that appear to be promising, despite the lack of long-term studies to confirm its lasting beneficial effects.

Clearly, morning bad breath in healthy subjects is a cosmetic problem analogous to body malodour. Products that claim to be effective cosmetics endure the scrutiny of the market place, and only those of merit should survive (Loesche 1999).

Even if the best beneficial effects on bad breath have been conferred by the use of chlorhexidine mouthrinses, confirmed by the percentage of changes in VSCs, the undesirable side effects associated with chlorhexidine recommend the usage of non-chlorhexidine mouthrinses. Nevertheless, long-term studies from candidate mouthrinses (such as essential oils, triclosan, cetylpyridinium chloride, zinc salts, chlorine dioxide or an oil-water-cetylpyridinium chloride mouthrinses) should be the aim for future researches (Kozlovsky et al. 1996).

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