# A comparison of bacterial growth inhibiting effects of six commercially available mouthrinses

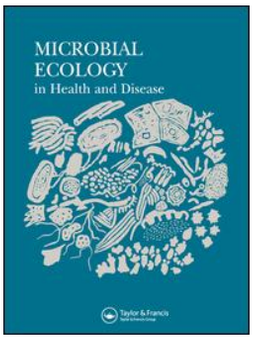
Perdok, J. F.; Van Der Mei, H. C.; Busscher, H. J.

*Published in:*
Microbial ecology in health and disease

*DOI:*
10.3109/08910608909140217



*Publication date:*
1989



Taylor & Francis
Taylor & Francis Group

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: https://www.tandfonline.com/loi/zmeh20

# A Comparison of Bacterial Growth Inhibiting Effects of Six Commercially Available Mouthrinses

J. F. Perdok, H. C. Van Der Mei & H. J. Busscher

**To cite this article:** J. F. Perdok, H. C. Van Der Mei & H. J. Busscher (1989) A Comparison of Bacterial Growth Inhibiting Effects of Six Commercially Available Mouthrinses, Microbial Ecology in Health and Disease, 2:3, 191-196, DOI: 10.3109/08910608909140217

**To link to this article:** https://doi.org/10.3109/08910608909140217



Published online: 11 Jul 2009.

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 2: 191–196 (1989)

# A Comparison of Bacterial Growth Inhibiting Effects of Six Commercially Available Mouthrinses

J. F. PERDOK*, H. C. VAN DER MEI and H. J. BUSSCHER

*Laboratory for Materia Technica, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.*

Received 17 November 1988; revised 18 February 1989

In this study the bacterial growth inhibiting effects of six commercially available mouthrinses (Hibident®, Prodent®, Merocet®, Listerine®, Veadent® and Meridol®) were determined. Hibident® was used as a positive control. Five strains were tested (*Streptococcus mutans* C67, *Streptococcus sanguis* CH3, *Veillonella alcalescens* V1, *Lactobacillus acidophilus* JP and *Actinomyces viscosus* C74), as representatives of the supragingival human microflora. The Maximal Inhibiting Dilution (MID) was measured in batch cultures for each product and strain. With respect to the positive control, Hibident® (containing 0·2 per cent chlorhexidine), the most effective product was Meridol® (containing 125 ppm aminefluoride 297 and 125 ppm stannous fluoride) followed by Merocet® (containing 0·05 per cent cetylpyridinium chloride), Veadent® (containing 0·03 per cent sanguinarine), Listerine® (containing phenolic compounds) and Prodent® (containing 0·5 per cent sodium fluoride). Although all products have been separately reported to yield a plaque reduction *in vivo*, this study provides a firm basis for a comparison between products, as they were all evaluated in a similar way.

KEY WORDS—Mouthrinses; Bacterial growth; Maximal Inhibiting Dilution.

## INTRODUCTION

The presence of dental plaque on teeth has been known since Anthony van Leeuwenhoek described in 1863 'little animals in the white matter' on his teeth. Its key role in the development of caries and periodontal disease is evident.[2,8] Dental plaque is described as a soft, predominantly bacterial material which develops on the surfaces of teeth and other oral surfaces.[9] *Streptococcus mutans*, lactobacilli and *Actinomyces viscosus* tend to colonise preferentially on the tooth surfaces.[5] Plaque removal is essential in preventing the development of carious lesions and gingival inflammation. In recent years mouthrinses containing antibacterial agents have been used to prevent plaque accumulation. Several compounds such as antibiotics, enzymes, quarternary ammonium compounds, phenolic compounds or bisbiguanides are used.[13] Antibiotics and enzymes are not appropriate for longterm plaque control for several reasons. Sensitisation of the patient or opportunistic infections can develop, making the antibiotic useless for more severe infections. In general the working mechanism of enzymes is too specific to render a good antiplaque effect.[11] Chlorhexidine is able to prevent plaque accumulation completely but has some undesirable side effects such as loss of taste and discoloration of teeth.[4] Hitherto, however, no other compound has been found with the same plaque preventing properties as chlorhexidine, despite the fact that numerous products have appeared on the market. Most of these products have been evaluated in separate studies for their effects on plaque prevention and inhibition of bacterial growth.[1,6,7,14,16,17] It is impossible, however, to make a real comparison of products on the basis of these studies due to differences in evaluation methods. Therefore, it is the aim of this study to compare the effects of six commercially available mouthrinses on the growth of five supragingival strains of oral bacteria which play an important role in the occurrence of dental caries and gingivitis.

## MATERIALS AND METHODS

### *Mouthrinses*

The mouthrinses employed in this study were all commercially obtained and used within three

*Author to whom correspondence should be addressed.

0891–060X/89/030191–06 $05.00

Table 1. Commercially purchased mouthrinses evaluated in this study together with their main active components and manufacturer

| Tradename | Main active component | Manufacturer |
|---|---|---|
| Hibident® | 0·2% chlorhexidine | ICI Dental Benelux, Belgium |
| Prodent® | 0·5% sodium fluoride | Intradal, The Netherlands |
| Merocet® | 0·05% cetylpyridinium chloride | Merrel Dow Pharmaceuticals, England |
| Listerine® | phenolic compounds | Lambert Chemical Co Ltd, England |
| Veadent® | 0·03% sanguinarine | Vipart Laboratories Inc, Collins USA |
| Meridol® | 125 ppm aminefluoride<br>125 ppm stannous fluoride | GABA Basel, Switzerland |

months after purchasing. Hibident®, a product based on chlorhexidine was included as a positive control. All products are listed in Table 1, together with their main additives and manufacturers.

*Bacterial strains*

The bacterial strains used in this study were selected to be representative for supragingival plaque and included *Streptococcus mutans* C67, *Steptococcus sanguis* CH3, *Veillonella alcalescens* V1, *Lactobacillus acidophilus* JP and *Actinomyces viscosus* C74. These strains were originally isolated from supragingival plaque from the human oral cavity and fresh isolates were frozen in small aliquots in Todd Hewitt broth (Oxoid) containing 7 per cent (v/v) dimethylsulfoxide at −20°C. At the start of the experiment bacteria were grown overnight at 37°C in an appropriate broth, being Todd Hewitt broth (Oxoid) for *S. mutans* C67 and *S. sanguis* CH3 and Brain Heart Infusion (Difco) for *L. acidophilus* JP and *A. viscosus* C74. For *V. alcalescens* V1, Todd Hewitt broth was supplemented with 1 per cent lactic acid. Anaerobic strains were cultured in an atmosphere of 80 per cent $CO_2$, 15 per cent $N_2$ and 5 per cent $H_2$. These cultures, having densities between $0{\cdot}5 \times 10^9$ and $1 \times 10^9$ cells/ml$^{-1}$, were used to inoculate second cultures containing various amounts of a mouthrinse.

*Determination of the growth inhibition*

Growth inhibiting effects of the mouthrinses for the various strains were first determined in a pilot study by adding widely varying amounts of a product to 10 ml bacterial culture. Different dilutions of the products were thus obtained and incubated again at 37°C for 18 h. Subsequently the extinction of the test tubes was measured in a photospectrometer (Bausch & Lomb Spectronic 20) at a wavelength of 660 nm employing a culture containing only the broth as a control. These results were applied to make appropriate dilution series for each product and strain based on one bacterial culture. The average extinctions of two such dilution series were plotted as a function of the dilution factor. The dilution factor, at which the extinction was 10 per cent below the control was taken as the Maximal Inhibiting Dilution (MID).

## RESULTS

Figure 1 shows examples for two products of the relation between the extinction and the dilution of a product. This figure includes two extreme examples; Hibident® inhibited growth up till approximately 2000 times dilution, whereas Prodent® only reduced growth in dilutions between 1 and 10. It should be noted that occasional, normal variations in the extinctions of the control occurred, due to differences in culture conditions. However, since each curve is based on one culture, this does not significantly affect the value for the MID's obtained. Also it can be seen from this figure that certain strains were more sensitive to a given product than others. For instance, *L. acidophilus* in the evaluation of Hibident® showed an extinction of the control of 0·6. The dilution factor at a 10 per cent reduced extinction can be read from Figure 1 to be

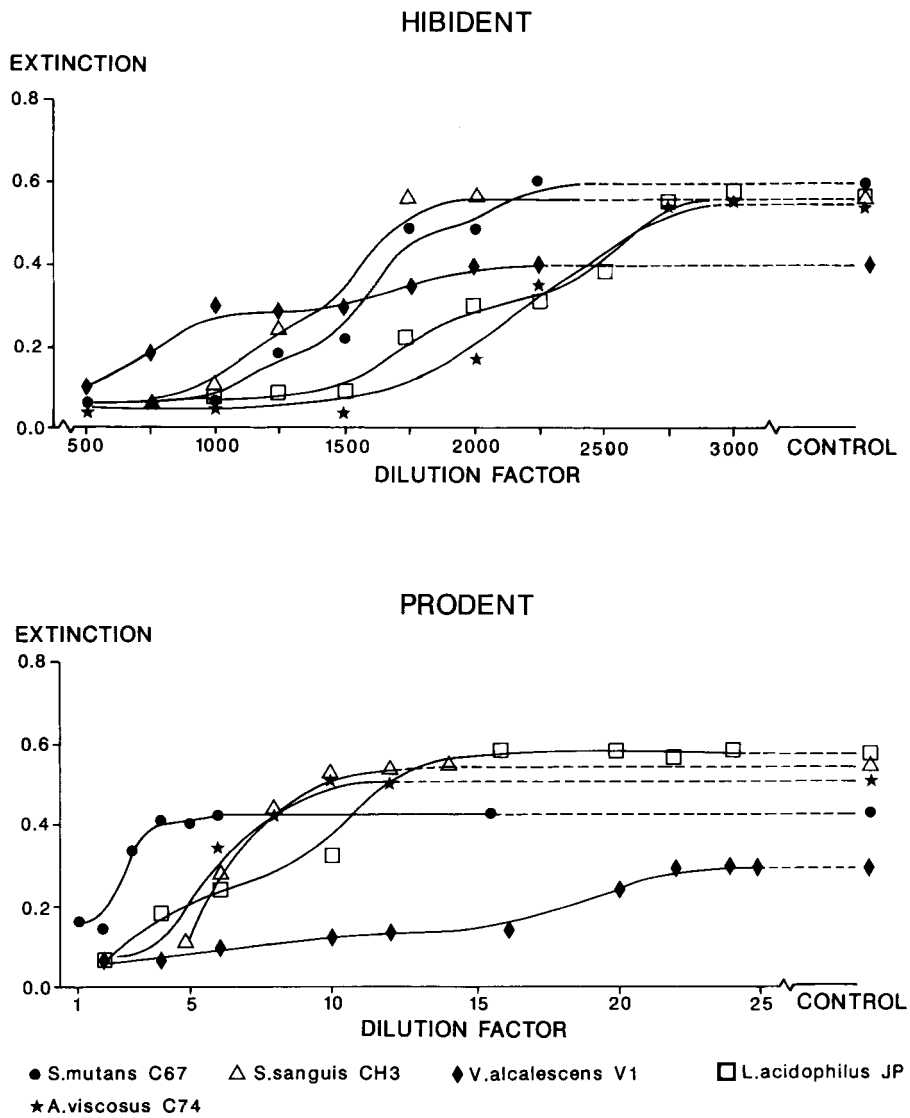



Figure 1. Examples of the relations between the extinctions and the dilution factors of two products averaged over two separate dilution series. Data for the control, containing only the broth, are indicated at infinite dilution

approximately 2750. MID's are listed in Table 2 for all strains and products. MID's derived from dilution series of separate bacterial cultures, never differed more than 4 per cent from the averages of two cultures presented in Table 2.

Subsequently, we defined a relative effectiveness (RE) of a product according to:

$$RE = \frac{\text{MID product}}{\text{MID Hibident®}} \times 100\% \qquad (1)$$

Figure 2 shows the relative effectiveness of the products for the various strains. As can be seen, only Merocet® and Meridol® approached the effectiveness of Hibident®, whereas Prodent®, Listerine® and Veadent® were hardly effective in reducing bacterial growth in comparison with Hibident®. Because the effectiveness of the products differed per strain, an average effectiveness ($\overline{RE}$) was calculated by averaging the values of RE according to equation (1) over all strains involved, thereby

Table 2. Maximal Inhibiting Dilution (MID) of six commercially available mouthrinses for various strains of oral bacteria from results of two separate runs

| Strain | Hibident® | Prodent® | Merocet® | Listerine® | Veadent® | Meridol® |
|---|---|---|---|---|---|---|
| *S. mutans* C67 | 1:1750 | 1:6 | 1:1000 | 1:12 | 1:60 | 1:1500 |
| *S. sanguis* CH3 | 1:1750 | 1:14 | 1:875 | 1:36 | 1:70 | 1:2000 |
| *V. alcalescens* V1 | 1:2000 | 1:22 | 1:500 | 1:50 | 1:100 | 1:2000 |
| *L. acidophilus* JP | 1:2750 | 1:12 | 1:625 | 1:90 | 1:50 | 1:1750 |
| *A. viscosus* C74 | 1:2750 | 1:10 | 1:1500 | 1:30 | 1:30 | 1:2500 |

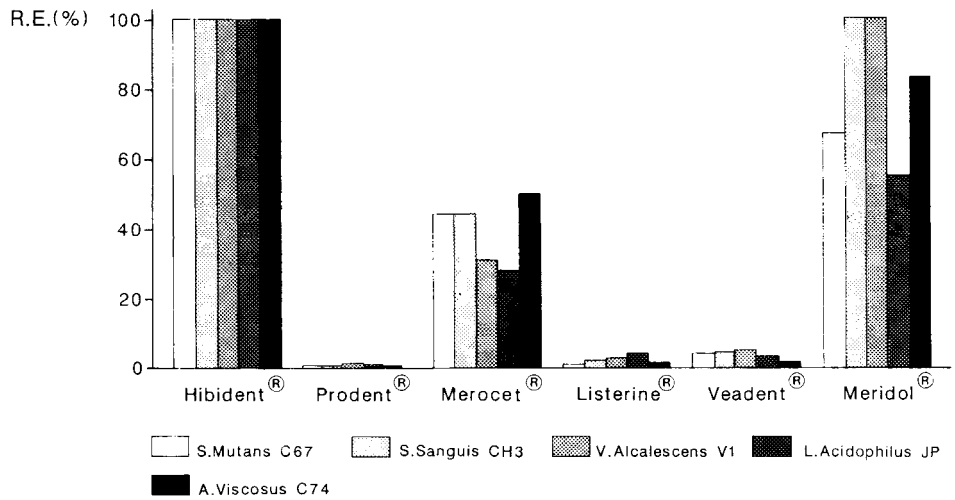



Figure 2. The relative effectiveness (RE) in inhibiting bacterial growth of six commercially available mouthrinses on five bacterial strains (Hibident® is set at 100 per cent)

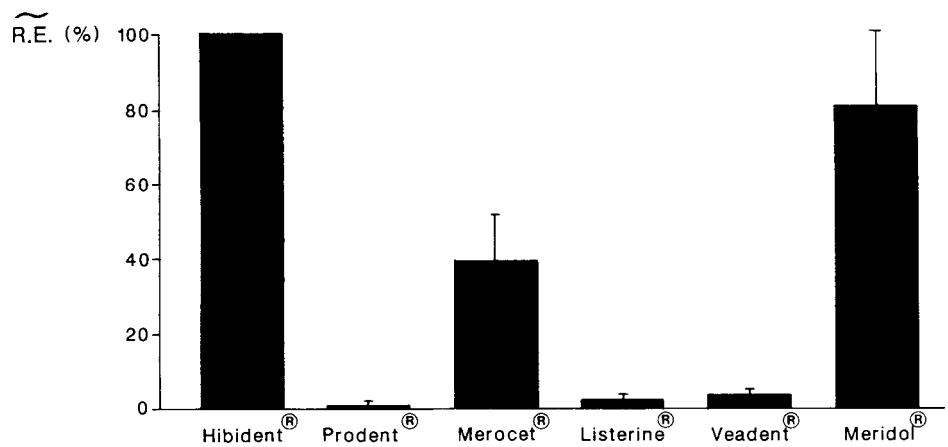



Figure 3. The average relative effectiveness ($\overline{RE}$) in inhibiting bacterial growth averaged over all strains involved of six commercially available mouthrinses on five bacterial strains (Hibident® is set at 100 per cent)

obtaining a relative effectiveness of the products ($\overline{RE}$) for the growth inhibiting effect on supragingival plaque. The $\overline{RE}$ for all products are presented in Figure 3.

## DISCUSSION

In this study we compared the *in vitro* effects of six commercially available mouthrinses on the growth of five different supragingival bacterial strains. All effects were measured with respect to Hibident®, which was used as a positive control. Thus we defined a relative effectiveness RE, based on the ratio between the Maximal Inhibiting Dilution (MID) of a product and of Hibident®.

For the evaluation of commercially available whole products an MID seems more appropriate than the Minimal Inhibiting Concentration (MIC) usually employed for the evaluation of a given component.[10] MID and MIC are however not directly comparable quantities as flavouring agents, colouring agents and surfactants all present in commercial products[12] can reduce the antibacterial properties of the active components.

Table 2 shows big differences between the sensitivities of the strains for a given product. The MID of Hibident® is extremely high for *L. acidophilus* JP, *A. viscosus* C74, *S. sanguis* CH3 and *S. mutans* C67. *V. alcalescens* V1 is relatively insensitive to Hibident®. The MID of Merocet® and Meridol® approach the values of Hibident®, although it should be noted that Meridol® is the only product significantly inhibiting the growth of *V. alcalescens* V1. Whereas Listerine® and Veadent® possess a low MID for all strains, Prodent® shows hardly any growth inhibiting effects. Prodent® however is not a product designed to have antibacterial effects, its major component being sodium fluoride, beneficial for enamel remineralisation.

Schaeken *et al.*[15] determined the composition of human plaque in volunteers who were treated once with a chlorhexidine gel. They found that *S. mutans* and *A. viscosus* were strongly suppressed, but *S. sanguis* was much less affected, which is in accordance with our *in vitro* results as far as *A. viscosus* is concerned (see Table 2).

Although some authors[18] report a significant plaque reduction in volunteers employing Veadent®, this study points to a negligible bacterial growth inhibiting effect of Veadent® compared to Hibident®, in correspondence with the clinical results of Abbas *et al.*[1] and Siegrist *et al.*[16]. Fine *et al.*[3] reported also a plaque reducing effect for Listerine®, which turned out to be very small compared to a chlorhexidine product.[13] Clinically significant effects of Meridol® and Merocet® have been reported by Perdok *et al.*[14] and Llewelyn[6] respectively.

In only one of the studies mentioned above were more than two products simultaneously evaluated,[15] undoubtedly due to the big strain extensive clinical trials put on human volunteers. Statistically significant plaque reductions of a product relative to a placebo however do not warrant any conclusions about the comparison with another product separately tested. All these factors contribute to the current controversies in the literature on the efficiency of several mouthrinses.

In general the plaque reducing effects of the various products predicted from this *in vitro* study (see Figure 3) correspond with separate *in vivo* effects reported, despite the fact that the activity of agents *in vivo* can be greatly affected, e.g. by a shorter duration of the action and the presence of saliva. As all products were evaluated here in an identical way, a much firmer basis for comparison is provided, yielding the conclusion that the bacterial growth inhibiting effect of Hibident® is the best, followed by Meridol® and Merocet®. Listerine®, Veadent® and Prodent® are of minor importance for inhibiting bacterial growth.

## ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr A. H. Weerkamp for his advice and help during the initiation of this study and to Mrs Marjon Schakenraad-Dolfing for manuscript preparation.

## REFERENCES

1. Abbas DK, Thrane P, Othman SJ. (1985). Effectiveness of Veadent as a plaque-inhibiting mouthwash. *Scandinavian Journal of Dental Research* **93,** 494–496.
2. Axelsson P, Lindhe J. (1978). Effect of controlled oral hygiene procedures on caries and periodontal disease in adults. *Journal of Clinical Periodontology* **5,** 133–151.
3. Fine DH, Letizia J, Mandel ID. (1985). The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. *Journal of Clinical Periodontology* **12,** 660–666.

4. Gjermo P. (1974). Chlorhexidine in dental praxis. *Journal of Clinical Periodontology* **1,** 143–152.
5. Houte van J. (1980). Bacterial adhesion in the mouth. In: Leach SA (ed) *Dental plaque and surface interactions*. IRL Press, London, pp. 69–100.
6. Llewelyn J. (1980). A double blind crossover trial on the effect of cetylpyridinium chloride 0·05% (Merocet) on plaque accumulation. *British Dental Journal* **148,** 103–104.
7. Löe M, Rindom Schiøtt C. (1970). The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *Journal of Periodontal Research* **5,** 79–83.
8. Löe M, Theillade E, Borglund Jensen S. (1965). Experimental gingivitis in man. *Journal of Periodontology* **36,** 177–187.
9. McHugh WD. (1986). Role of supragingival plaque in oral disease initiation and progression. State-of-the-science review. In: Löe M, Kleinman DV, (eds) *Dental plaque control measures and oral hygiene practices*. IRL Press, Oxford, Washington DC, pp. 1–12.
10. Nolte WA. (1982). *Oral Microbiology*. 4th edition. The C.V. Mosby Company, St Louis, p. 100.
11. Nyman S, Lindhe J, Janson JC. (1972). The effects of a bacterial dextranase on human dental plaque formation and gingivitis development. *Odontologisk Revy* **23,** 243–252.
12. Pader M. (1982). Surfactants in oral hygiene products. In: Rieger MM (ed) *Surfactants in cosmetics*. Marcel Dekker Inc., New York, pp. 293–348.
13. Perdok JF, Dijk van LJ, Busscher HJ, Arends J, Poel van de ACM. (1988). Plaquepreventie door mondspoelmiddelen. *Nederlands Tijdschrift voor Tandheelkunde* **95,** 8–14.
14. Perdok JF, Busscher HJ, Weerkamp AH, Arends J. (1988). The effect of an aminefluoride-stannous fluoride containing mouthrinse on surface free energy and the development of plaque and gingivitis. *Clinical and Preventive Dentistry* **10,** 3–9.
15. Schaeken MJM, Jong de MH, Franken HCM, Hoeven van der JS. (1984). Effect of chlorhexidine and iodine on the composition of the human dental plaque flora. *Caries Research* **18:** 401–407.
16. Siegrist BE, Gusberti FA, Breckx MC, Weber HP, Lang NP. (1986). Efficacy of supervised rinsing with chlorhexidine digluconate in comparison to phenolic and plant alkaloid compounds. *Journal of Periodontal Research* **21,** suppl 16: 60–73.
17. Tinanoff N, Hock J, Camosci D, Hellden L. (1980). Effect of stannous fluoride mouthrinse on dental plaque formation. *Journal of Clinical Periodontology* **7,** 232–241.
18. Wennström J, Lindhe J. (1985). Some effects of a sanguinarine containing mouthrinse on developing plaque and gingivitis. *Journal of Clinical Periodontology* **12,** 867–872.