Evaluation of the Antimicrobial Activity of Dentifrices on Human Oral Bacteria

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Abstract

- **Objective:** *In vitro* testing of antimicrobial agents is an important tool in the testing hierarchy, and may provide interesting insights into their potential clinical efficacy. Agents with demonstrable *in vitro* antimicrobial activity may be effective against the same microorganisms *in vivo*, whereas agents without demonstrable *in vitro* antimicrobial activity are unlikely to exhibit *in vivo* antimicrobial activity. In addition, these methods may also be useful in screening antimicrobial agents in product formulations because such agents with both *in vitro* and *in vivo* activity may have reduced antimicrobial effects when formulated into a dentifrice. Accordingly, this study examined the *in vitro* and *ex vivo* antimicrobial activity of three commercial dentifrices: one formulated with 0.243% sodium fluoride (Crest® Cavity Protection Toothpaste-Regular); one with 0.454% stannous fluoride, sodium hexametaphosphate, and zinc lactate (Crest® Pro-Health®), and one with 0.3% triclosan, 2.0% PVM/MA copolymer, and 0.243% sodium fluoride (Colgate® Total®).
- **Methods:** The minimum inhibitory concentration (MIC) of each dentifrice was determined for resident oral bacterial species, including bacteria that are associated with dental caries, periodontitis, and oral halitosis. Evaluations were performed on individual laboratory strains, and on oral bacteria from supragingival plaque samples obtained from 10 adults and from oral rinse samples obtained from 18 adults.
- **Results:** The lowest MICs against the oral strains and human samples, *i.e.*, greatest antimicrobial activity, were seen for the triclosan/copolymer dentifrice. There was, in general, a four-fold difference in MICs between the triclosan/copolymer dentifrice and the stannous fluoride/sodium hexametaphosphate/zinc lactate dentifrice. The triclosan/copolymer dentifrice significantly inhibited periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, and *Fusobacterium nucleatum*. In *ex vivo* tests measuring antimicrobial effects, the triclosan/copolymer dentifrice substantially inhibited bacterial growth after 30-, 60-, and 120-second exposures compared to the sodium fluoride or stannous fluoride/sodium hexametaphosphate/zinc lactate dentifrices. Similarly, in *ex vivo* tests measuring antimicrobial effects on supragingival plaque biofilms, the triclosan/copolymer dentifrice substantially inhibited bacterial growth compared to the other test dentifrices.
- **Conclusion:** Different *in vitro* and *ex vivo* analyses show that the triclosan/copolymer dentifrice has significant antimicrobial activity on oral bacteria, including species causing dental caries, periodontitis, and oral halitosis, and it provides superior efficacy compared to the stannous fluoride/sodium hexametaphosphate/zinc lactate dentifrice.

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Introduction

A variety of microbiological techniques have been used to identify and characterize the microorganisms residing in the human oral cavity. This is an important activity in order to further knowledge of the microorganisms that colonize the human body, the microbiome,[1] because oral microorganisms can cause dental caries and periodontal disease, the most common infectious diseases in man. Ninety-two percent of people in the US 65 years of age and older have dental caries in their permanent teeth,[2] 50.3% of people in the US population 30 years or older have gingivitis,[3] and 26% have destructive periodontitis.[4] Microscopy, bacterial culture, and, most recently, nucleic acid sequencing are routinely used to identify microorganisms.[5] Microbial susceptibility to antimicrobial agents is routinely evaluated by disk diffusion assays (Kirby-Bauer), broth and agar dilution assays, and combination assays, such as the spiral gradient endpoint method and E test.[5,6]

Microbial susceptibility tests have obvious clinical applications in the prevention and treatment of infectious diseases.[5,6] Of particular interest is the correlation between *in vitro* antimicrobial testing and *in vivo* efficacy. An agent that does not exhibit *in vitro* antimicrobial activity is unlikely to demonstrate *in vivo* antimicrobial activity. On the other hand, an antimicrobial agent that demonstrates significant *in vitro* antimicrobial activity may or may not exert similar levels of *in vivo* antimicrobial activity. These levels are typically expressed as the minimum inhibitory concentration (MIC) or the minimum bactericidal concentration (MBC).[7] The MIC is the lowest concentration of a drug that inhibits growth, while the MBC is the lowest concentration that kills a microorganism. MICs are used to determine susceptibility or resistance of microorganisms to an antimicrobial agent, *i.e.*, what kind and how much of an antimicrobial agent to use in a particular clinical situation. Antibiotic breakpoints are defined based on the MIC and on the pharmacokinetics in healthy volunteers.[5,6]

In this study, the MICs for commercial dentifrices formulated with stannous fluoride/sodium hexametaphosphate/zinc lactate, triclosan/copolymer/sodium fluoride, and sodium fluoride were determined for microorganisms commonly found in the human oral cavity, using both laboratory strains and samples obtained from adult subjects to determine the effects of different treatment durations on microbial viability.

Materials and Methods

Media, Chemicals, and Reagents

Bacteriological media were obtained from Becton-Dickinson (Sparks, MD, USA) and formulated in accordance with manufacturer's instructions. Buffers, chemicals, and laboratory reagents were obtained from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise indicated.

Bacterial Strains

Oral bacteria were obtained from either the American Type Culture Collection (Manassas, VA, USA) or from the University at Buffalo School of Dental Medicine (Buffalo, NY, USA), and included oral and non-oral bacteria that can cause periodontal disease, dental caries, or oral halitosis. All bacteria were cultured on enriched tryptic soy agar, supplemented with 5% defibrinated sheep blood, 5.0 µg/mL hemin, and 0.5 µg/mL vitamin K_1.

Dentifrices

Commercially available dentifrices for this investigation included a 0.243% sodium fluoride toothpaste (Crest® Cavity Protection Toothpaste-Regular, Procter & Gamble, Cincinnati, OH, USA; henceforth F), a 0.454% stannous fluoride, sodium hexametaphosphate, and zinc lactate toothpaste (Crest Pro-Health®, Procter & Gamble, Cincinnati, OH, USA; henceforth SnF_2), and a toothpaste containing 0.3% triclosan, 2.0% polyvinylmethyl ether maleic acid (PVM/MA) copolymer, and 0.243% sodium fluoride (Colgate® Total®, Colgate-Palmolive Company, New York, NY; henceforth TCN/C).

Laboratory Tests

Bacteria were tested against the F, SnF_2, and TCN/C toothpastes, each dispersed in sterile water. Various dilutions of toothpaste slurries were incubated with bacteria cultured in liquid media, and the MIC was defined as the lowest concentration (highest dilution) in which the bacteria failed to grow. Positive controls included bacteria without toothpaste slurry, and negative controls included toothpaste slurry without added bacteria.

Ex Vivo *Tests*

Supragingival plaque was collected from 10 adults, dispersed by sonication, and distributed onto solid media containing defibrinated sheep blood with different concentrations of toothpaste. Following anaerobic incubation at 37°C for five days, the number of viable bacteria (CFU/mL) were enumerated from the solid media. Oral rinse samples were obtained from 18 adults following informed consent and a one-week "washout" with a commercially available fluoride dentifrice. The subjects rinsed with 10 mL of sterile water for 10 seconds and expectorated into sterile tubes. The oral rinse samples were mixed with toothpaste slurry for 30, 60, or 120 seconds, and distributed onto solid media containing defibrinated sheep blood.

The study protocol was approved by the Health Sciences Institutional Review Board at the University at Buffalo.

Statistical Analysis

Viable microorganisms recovered after antimicrobial treatments were evaluated by ANOVA and Tukey multiple comparison tests, with subjects and dentifrice in the model. Treatment effects are reported as significant at $p < 0.05$.

Results

The TCN/C dentifrice demonstrated significantly higher antimicrobial activity (Table I) than the other two dentifrices, with MICs to oral bacteria ranging from less than 0.94 µg/mL to 30 µg/mL. By comparison, there were higher MICs for the SnF_2 dentifrice, ranging from 1.8 to 75 µg/mL. There was especially notable antimicrobial activity for the TCN/C dentifrice toward periodontal pathogens, including *Aggregatibacter actinomycetemcomitans*, *Campylobacter*, *Eikenella corrodens*, and *Fusobacterium nucleatum*. For the SnF_2 dentifrice, the highest MICs were to *Capnocytophaga gingivalis* and *Actinomyces meyerii,* but were otherwise similar to the F dentifrice for most of the gram-positive and gram-negative test microorganisms.

Table I
Minimum Inhibitory Dentifrice Concentrations

Bacterial Species	Strain Number	Colgate Total	Crest Pro-Health	Crest Cavity Protection-Regular
Oral and Non-oral Microorganisms				
Actinomyces meyerii	ATCC 33972	15	75	30
Actinomyces viscosus	ATCC 43146	7.5	7.5	30
Bacillus cereus	ATCC 11778	7.5	15	30
Bacillus subtilis	ATCC 6051	15	>150	75
Candida albicans	ATCC 90028	30	150	75
Escherichia coli	ATCC 4157	7.5	150	>150
Moraxella catarrhalis	ATCC 8176	< 0.94	3.5	7.5
Staphylococcus aureus	ATCC 6538	15	30	30
Veillonella dispar	ATCC 17748	15	30	75
Veillonella atypica	ATCC 27215	7.5	7.5	15
Periodontal Pathogens				
Aggregatibacter actinomycetemcomitans	ATCC 43717	< 0.94	3.5	30
Aggregatibacter actinomycetemcomitans	ATCC 43718	1.8	3.5	30
Capnocytophaga gingivalis	ATCC 33124	3.5	75	15
Campylobacter rectus	ATCC 33238	1.6	7.5	15
Eikenella corrodens	ATCC 23834	< 0.94	15	30
Fusobacterium nucleatum	ATCC 25586	1.8	7.5	15
Porphyromonas gingivalis	ATCC 53977	1.8	1.8	15
Prevotella intermedia	ATCC 25611	3.5	3.5	15
Prevotella melanogenica	ATCC 25845	3.5	3.5	30
Prevotella nigresence	NCTC 9336	1.8	1.8	15
Cariogenic Bacteria				
Streptococcus mutans	ATCC 6538	7.5	7.5	30
Streptococcus gordonii	ATCC 10558	3.5	7.5	15
Oral Halitosis-Causing Bacteria				
Solobacterium moorei	J10654	30	30	30

Figure 1 shows individual reductions in supragingival plaque bacteria for each active formulation compared to the F dentifrice in 10 subjects. For all subjects, the greatest reductions were for the TCN/C dentifrice compared to the SnF_2 dentifrice. In the latter, five of 10 subjects showed increases in the number of supragingival plaque bacteria as compared to the F dentifrice. Treatment with the TCN/C dentifrice demonstrated significant reductions in the number of supragingival plaque bacteria from all samples when compared to the F dentifrice.

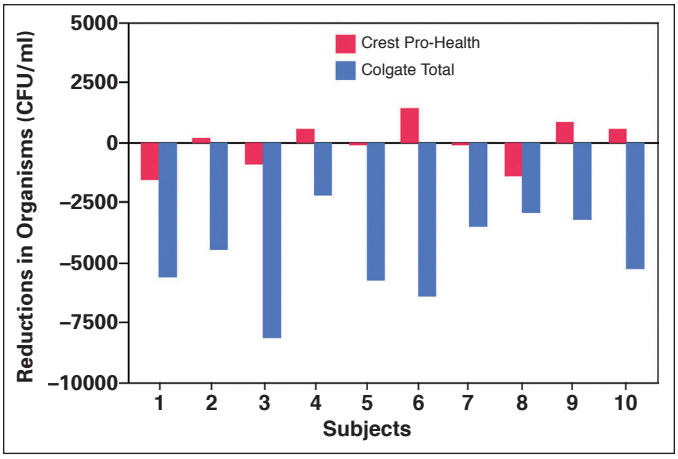

Figure 1. *Effect of treatment by Crest Pro-Health and Colgate Total on supragingival plaque bacteria in comparison to Crest Cavity Protection Toothpaste-Regular. Bars indicate reductions (a negative value) or increments (a positive value) in viable organisms (× 10^4 CFU/ml) from 10 subjects.*

Figure 2 shows the average reduction in the number of supragingival plaque bacteria for each test dentifrice compared to the F dentifrice for the 10 test subjects. For the SnF_2 dentifrice group, the average reduction was less than 3×10^5 viable colony forming units per ml, while for the group using the TCN/C dentifrice the average reduction was greater than 4.5×10^7 colony forming units per ml.

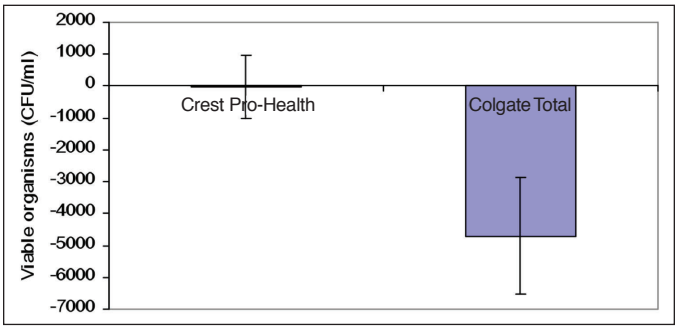

Figure 2. *Average reductions in supragingival plaque bacteria for Crest Pro-Health and Colgate Total from the Crest Cavity Protection Toothpaste-Regular toothpaste. Graph indicates average reductions ± standard deviation (× 10^4 CFU/ml) from 10 subjects.*

Figure 3 shows the log transformed number of colony forming units per ml recovered after treatment with the F, SnF_2, or TCN/C dentifrices for the 10 test subjects; that is, the remaining number of supragingival plaque bacteria. The group treated with the TCN/C dentifrice exhibited the lowest mean number of remaining supragingival plaque bacteria, approximately 6.7 Log_{10} CFU/ml, while those treated with the SnF_2 and the F dentifrices exhibited approximately 7.7 Log_{10} CFU/ml after treatment.

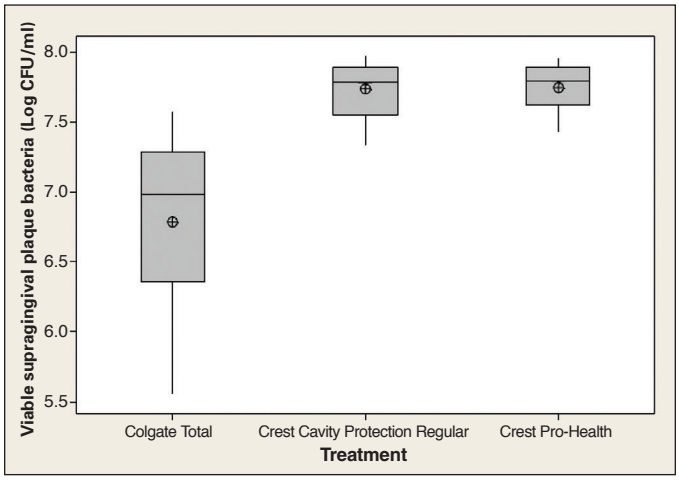

Figure 3. *Effect of treatment with Crest Cavity Protection Toothpaste-Regular, Crest Pro-Health, or Colgate Total on supragingival plaque bacteria from 10 subjects. Box-plots indicate viable supragingival plaque bacteria recovered (Log CFU/ml) after each treatment. Horizontal line in box-plot is the median and symbol represents the average.*

Average microbial viability after 30-, 60-, and 120-second exposures of oral rinse samples from the 18 adults is shown in Figures 4–6. After thirty seconds of dentifrice treatment (Figure 4) on oral bacteria collected from 18 adult volunteers, the lowest numbers of viable bacteria were seen in the samples treated with the TCN/C dentifrice, approximately 5.2 Log_{10} CFU/ml compared to approximately 6.1 Log_{10} CFU/ml and 6.1 Log_{10} CFU/ml for the SnF_2 and the F dentifrices, respectively. For sixty seconds of dentifrice treatment (Figure 5), the lowest numbers of viable bacteria were also seen in the samples treated with the TCN/C dentifrice, approximately 5.2 Log_{10} CFU/ml compared to approximately 6.1 Log_{10} CFU/ml and 5.8 Log_{10} CFU/ml for the SnF_2 and the F dentifrices, respectively. For 120 seconds of dentifrice treatment (Figure 6), the lowest numbers of viable bacteria were again seen in the samples treated with the TCN/C dentifrice, approximately 4.6 Log_{10} CFU/ml compared to approximately 5.9 Log_{10} CFU/ml and 5.5 Log_{10} CFU/ml for the SnF_2 and F dentifrices, respectively.

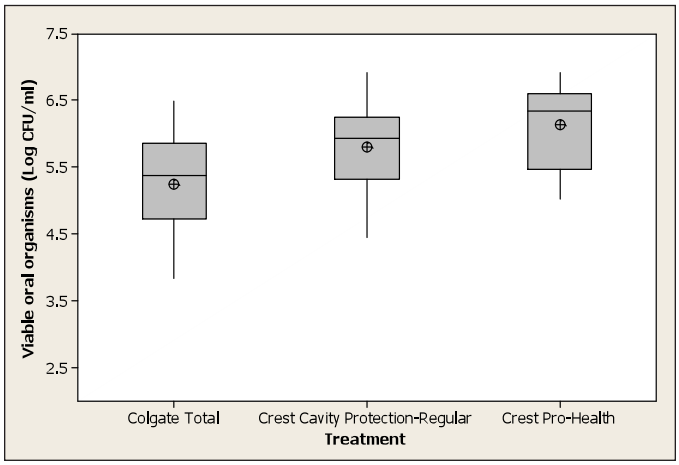

Figure 4. *Effect of 30-second dentifrice treatments on oral organisms. Box-plots indicate viable organisms recovered (Log CFU/ml) from 18 subjects after each treatment. Horizontal line in box-plot is the median and symbol represents the average.*

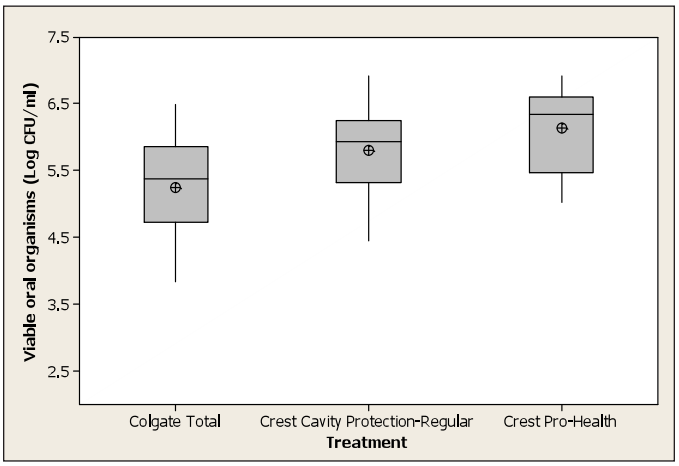

Figure 5. *Effect of 60-second dentifrice treatments on oral organisms. Box-plots indicate viable organisms recovered (Log CFU/ml) from 18 subjects after each treatment. Horizontal line in box-plot is the median and symbol represents the average.*

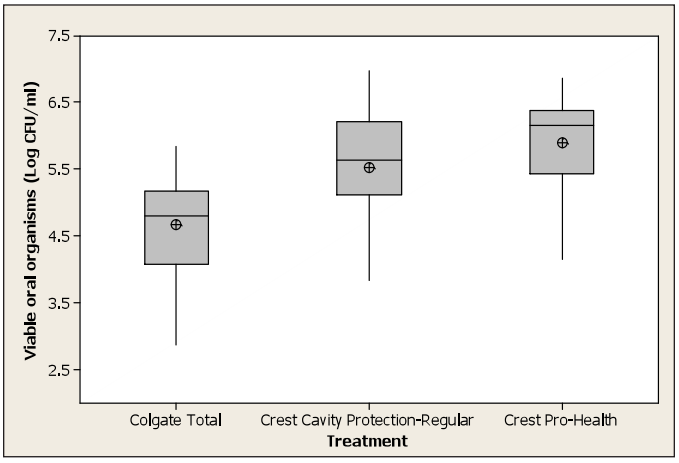

Figure 6. *Effect of 120-second dentifrice treatments on oral organisms. Box-plots indicate viable organisms recovered (Log CFU/ml) from 18 subjects after each treatment. Horizontal line in box-plot is the median and symbol represents the average.*

Two-way ANOVA at 30-, 60-, and 120-second treatment durations of oral rinse samples showed subjects and dentifrices were statistically significant ($p < 0.0005$). The TCN/C dentifrice was more effective than the other two dentifrices at both the 30- and 60-second post-treatment assessments ($p < 0.0005$), and the F dentifrice more effective than the SnF_2 dentifrice ($p = 0.0001$) at the 60-second post-treatment assessment. The TCN/C dentifrice was more effective at 120 seconds post-treatment than the other two dentifrices ($p < 0.0005$), but the F dentifrice was not significantly different from the SnF_2 dentifrice ($p = 0.058$).

Two-way ANOVA with subject and dentifrice as effects was used to examine microbial viability of the supragingival plaque samples following treatment, and while each dentifrice demonstrated significant effects ($p < 0.0005$), the TCN/C dentifrice demonstrated the greatest effect. It also demonstrated significantly higher bacterial growth inhibition by *post hoc* Tukey multiple comparison tests than the other dentifrices ($p < 0.0005$). There was no significant difference between the SnF_2 and F dentifrices ($p = 0.99$).

Discussion

This study examined the *in vitro* antimicrobial effects of three different commercially available toothpastes utilizing laboratory strains of oral and non-oral bacteria, and bacteria from supragingival plaque samples and oral rinse samples from adult volunteers. Use of well-characterized laboratory strains enables comparisons between antimicrobial tests performed at different times in the same laboratory, or performed in different laboratories.[6] The use of bacteria obtained from supragingival plaque samples and from oral rinse samples recently obtained from human volunteers facilitates testing of "wild-type" bacterial strains that often differ significantly from laboratory strains in terms of virulence factors and pathogenicity.[8] Bacteria in biofilms, such as dental plaque, are much less susceptible to antimicrobial agents, and demonstrate considerable physiologic variations within their organized structure compared to planktonic organisms.[8,9] For example, fresh isolates of *Aggregatibacter actinomycetemcomitans* grow as rough adherent colonies in broth media, while laboratory strains grow as smooth, non-adherent colonies.[10] Even strains of the same bacterial species isolated at the same time from the same individual, such as *Veillonella*, *Porphyromonas gingivalis* and streptococci, differ with regard to antimicrobial susceptibility and virulence.[11,12] The use of oral rinse and dental plaque samples also facilitates identification of bacteria with intermediate susceptibility, or that are resistant to specific concentrations of dentifrice incorporated into the media. Nonetheless, *in vitro* testing in the present study was consistent in demonstrating significantly greater *in vitro* activity of the triclosan-containing dentifrice compared to the other two toothpastes, and in predicting clinical efficacy in inhibiting dental plaque and other oral bacteria. Differences between the F and SnF_2 toothpastes were less notable at post-treatment evaluations.

The prediction of clinical efficacy derived from the *in vitro* testing has been confirmed in a number of published clinical trials showing that Colgate Total reduces dental plaque and associated gingival inflammation. Fifteen published studies involving 2,500 patients[7,13-26] found that Colgate Total was highly effective in reducing dental plaque and gingivitis. Reductions in dental plaque are reported to be as high as 59%, while reductions in gingivitis are reported to be as high as 51% compared to a regular fluoride dentifrice. For example, a random, double-blind, three-year clinical study by Rosling, *et al*.,[16] examining the subgingival microbiota of adults with recurrent advanced periodontitis, found a significant reduction in the number of viable bacteria and probing pocket depth in subjects using Colgate Total. A random, double-blind, clinical study by Deasy, *et al*.,[17] examining the effect of Colgate Total on plaque and gingivitis in 139 subjects, found a nearly 19% reduction in supragingival plaque and a 17% reduction in gingivitis in 139 subjects after three months of use, and a 32% reduction in supragingival plaque and a 26% reduction in gingivitis in 121 subjects after six months of use. A six-month, double-blind clinical study by Bolden, *et al*.[24] of 325 subjects found a 17% reduction in plaque and a 29% reduction in gingival inflammation in 155 subjects using Colgate Total, compared to 155 subjects using a control dentifrice. Thus for TCN/C, *in vitro* antimicrobial testing, demonstrating the inhibition of oral bacteria, was predictive of significant clinical

efficacy in inhibiting dental plaque and plaque-related gingival inflammation in human subjects.

Summary

Colgate Total, a dentifrice containing 0.3% triclosan, 2.0% copolymer, and 0.243% sodium fluoride, demonstrated the best broad-spectrum antimicrobial activity, that was approximately four-fold better than Crest Cavity Protection Toothpaste-Regular, a dentifrice formulated with 0.243% sodium fluoride, and Crest Pro-Health, a dentifrice formulated with 0.454% stannous fluoride, sodium hexametaphosphate, and zinc lactate. Colgate Total significantly inhibited a variety of oral bacteria, including periodontal pathogens such as *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, and *Fusobacterium nucleatum*, cariogenic bacteria such as mutans streptococci, and bacteria-causing oral halitosis such as *Solobacterium moorei*. In addition to its broad-spectrum antimicrobial activity against laboratory strains of bacteria, Colgate Total demonstrated a substantially greater broad-spectrum inhibition of bacteria from oral rinse and dental plaque samples from adult subjects when compared to the Crest Cavity Protection Toothpaste-Regular or Crest Pro-Health toothpastes.

In summary, results from *in vitro* and *ex vivo* testing demonstrate that Colgate Total had significantly better antimicrobial activity on oral bacteria, including species causing dental caries, periodontitis, and oral halitosis.

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References

1. NIH Human Microbiome Project, http://nihroadmap.nih.gov/hmp/.
2. NIDCR website, Dental caries in permanent (adult) teeth. http://www.nidcr.nih.gov/DataStatistics/FindDataByTopic/DentalCaries/DentalCariesAdults20to64.htm.
3. NIDCR/CDC Dental, Oral and Craniofacial Data Resource Center, Oral Health, U.S. 2002 Annual Report, Section 3: Periodontal disease. http://drc.hhs.gov/report/3_1.htm.
4. NIDCR/CDC Dental, Oral and Craniofacial Data Resource Center, Data Tables for Oral Health Indicators, http://drc.hhs.gov/report/dqs_tables/dqs_3_2_1.htm.
5. Murray PR. *Manual of Clinical Microbiology*, 9th Ed. ASM Press, Herndon, VA, 2007.
6. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009;49:1749-1755.
7. Garcia-Godoy F, Garcia-Godoy F, DeVizio W, Volpe AR, Ferlauto RJ, Miller JM. Effect of a triclosan/copolymer/fluoride dentifrice on plaque formation and gingivitis: A 7-month clinical study. *Am J Dent* 1990;3:S15-S26.
8. Fux CA, Shirtliff M, Stoodley P, Costerton JW. Can laboratory reference strains mirror "real-world" pathogenesis? *Trends Microbiol* 2005;13:58-63.
9. Schaudinn C, Gorur A, Keller D, Sedghizadeh PP, Costerton JW. Periodontitis: An archetypical biofilm disease. *J Am Dent Assoc* 2009;140:978-986.
10. Zambon JJ. *Actinobacillus actinomycetemcomitans* in human periodontal disease. *J Clin Periodontol* 1985;12:1-20.
11. Jain S, Darveau RP. Contribution of porphyromonas gingivalis lipopolysaccharide to periodontitis. *Periodontol 2000* 2010;54:53-70.
12. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Community Dent Oral Epidemiol* 2005;33:248-255.
13. Gaffar A, Nabi N, Kashuba B, Williams M, Herles S, Olsen S, Afflitto J. Antiplaque effects of dentifrices containing triclosan/copolymer/NaF system versus triclosan dentifrices without the copolymer. *Am J Dent* 1990;3:S7-S14.
14. Denepitiya JL, Fine D, Singh S, DeVizio W, Volpe AR, Person P. Effect upon plaque formation and gingivitis of a triclosan/copolymer/fluoride dentifrice: A 6-month clinical study. *Am J Dent* 1992;5:307-311.
15. Sharma NC, Galustians HJ, Qaquish J, Galustians A, Rustogi KN, Petrone ME, Chaknis P, Garcia L, Volpe AR, Proskin HM. The clinical effectiveness of a dentifrice containing triclosan and a copolymer for controlling breath odor measured organoleptically twelve hours after tooth brushing. *J Clin Dent* 1999;10:131-134.
16. Rosling B, Dahlén G, Volpe A, Furuichi Y, Ramberg P, Lindhe J. Effect of triclosan on the subgingival microbiota of periodontitis-susceptible subjects. *J Clin Periodontol* 1997;24:881-887.
17. Deasy MJ, Singh SM, Rustogi KN, Petrone DM, Battista G, Petrone ME, Volpe AR. Effect of a dentifrice containing triclosan and a copolymer on plaque formation and gingivitis. *Clin Prev Dent* 1991;13:12-19.
18. Mankodi S, Walker C, Conforti N, DeVizio W, McCool JJ, Volpe AR. Clinical effect of a triclosan-containing dentifrice on plaque and gingivitis: A six-month study. *Clin Prev Dent* 1992;14:4-10.
19. Svatun B, Saxton CA, Huntington E, Cummins D. The effects of three silica dentifrices containing triclosan on supragingival plaque and calculus formation and on gingivitis. *Int Dent J* 1993;43:441-452.
20. Palomo F, Wantland L, Sanchez A, Volpe AR, McCool J, DeVizio W. The effect of three commercially available dentifrices containing triclosan on supragingival plaque formation and gingivitis: a six month clinical study. *Int Dent J* 1994;44:75-81.
21. Barnes VM, Richter R, Vandeven M, Xu T, DeVizio W. Clinical investigation of the antiplaque efficacy of a new variant of a commercially available triclosan/copolymer/fluoride dentifrice. *J Clin Dent* 2008;19:81-84.
22. Mateu FA, Boneta AE, DeVizio W, Stewart B, Proskin HM. A clinical investigation of the efficacy of two dentifrices for controlling established supragingival plaque and gingivitis. *J Clin Dent* 2008;19:85-94.
23. Cubells AB, Dalmau LB, Petrone ME, Chaknis P, Volpe AR. The effect of a triclosan/copolymer/fluoride dentifrice on plaque formation and gingivitis: A six-month clinical study. *J Clin Dent* 1991;2:63-69.
24. Bolden TE, Zambon JJ, Sowinski J, Ayad F, McCool JJ, Volpe AR, DeVizio W. The clinical effect of a dentifrice containing triclosan and a copolymer in a sodium fluoride/silica base on plaque formation and gingivitis: A six-month clinical study. *J Clin Dent* 1992;3:125-131.
25. Renvert S, Birkhed D. Comparison between 3 triclosan dentifrices on plaque, gingivitis and salivary microflora. *J Clin Periodontol* 1995;22:63-70.
26. Triratana T, Rustogi KN, Volpe AR, DeVizio W, Petrone M, Giniger M. Clinical effect of a new liquid dentifrice containing triclosan/copolymer on existing plaque and gingivitis. *J Am Dent Assoc* 2002;133:219-225.
27. Hu D, Zhang J, Wan H, Zhang Y, Volpe AR, Petrone ME. Efficacy of a triclosan/copolymer dentifrice in the control of plaque and gingivitis: A six-month study in China. *Hua Xi Kou Qiang Yi Xue Za Zhi* 1997;15:333.