Antiplaque biocides and bacterial resistance: a review


Abstract
Modern dentistry emphasizes the importance of dental plaque control to improve oral health. The use of oral care formulations with antiplaque biocides plays a crucial role in patient-directed approaches for plaque control. The antiplaque efficacies of these formulations have been extensively studied in many long-term clinical studies designed in accordance with well-accepted guidelines. The results from these studies conclusively demonstrate that long-term use of oral care formulations with well-known antiplaque biocides such as chlorhexidine and triclosan reduce supragingival plaque and gingivitis. This review summarizes microbiological results from clinical studies conducted with oral care formulations containing antiplaque biocides. Results from a number of long-term clinical studies conducted under real-life use conditions indicate no adverse alterations in the bacteria found in dental plaque or emergent microbial resistance. Additionally, microbial sampling of dental plaque subsequent to extended use of antiplaque biocides reveals no increase in resistant microflora. Large numbers of common oral bacteria isolated from patients using chlorhexidine indicate no increase in microbial resistance to chlorhexidine or to commonly used antibiotics. The effects of antiplaque biocides containing oral care formulations on dental plaque that exists naturally as a biofilm are examined. These formulations contain biocide, surfactants, polymers and other components that are effective against the biofilm. In summary, the results of studies on the real-life use of oral care formulations with antiplaque biocides show no emergence of resistant microflora or alterations of the oral microbiota, while such formulations have been found to provide the benefits of reducing plaque and gingivitis.

Biocides comprise a large group of diverse chemical agents that are effective in inactivating a variety of microorganisms. Because of their long history of safety, biocides find utility in diverse areas (Denyer 1995, McDonnell & Russell 1999) for a variety of applications. For example, they are used as antiseptics and disinfectants in hospitals, formulated in rinses or gels for prescription by dentists, and used in public health for water purification and in consumer care products, including oral care formulations (Bloomfield 1996, Eley 1999, McDonnell & Russell 1999, Rutala et al. 2000, Slots 2000, White & McDermott 2001). Some commonly used biocides and their applications are summarized in Table 1.

This article reviews the use of biocides for antiplaque effects in oral care formulations, with an emphasis on long-term clinical studies that examine the effects of antiplaque biocide formulations on the bacteria of dental plaque. Results from long-term microbiological monitoring studies examining the oral flora for biocide resistance following extended use of oral care formulations with antiplaque biocides are presented. In addition, the antibiotic susceptibility of large numbers of clinical strains of common oral bacteria obtained from subjects following the use of chlorhexidine formulations is discussed. Antiplaque oral care formulations contain a variety of other components such as surfactants and antinucleating agents (which prevent mineralization of soft plaque). These formulations inhibit the formation of the supragingival dental plaque microbial biofilm. Recent laboratory and clinical evidence that demonstrates the inhibition of bacteria in the dental plaque biofilm by oral care formulations is examined. Another issue of considerable importance is the high concentration of biocides in form-
mulations (Russell & McDonnell 2000). The typical in-use concentrations of these biocides are substantially higher than is required to inhibit microorganisms. Taken together, these results indicate that microbial resistance is unlikely to occur under normal usage conditions of oral care formulations with antiplaque biocides.

The discussions in this review are with reference to clinical studies conducted with biocides in oral care formulations, including those used for hand disinfection in dental clinics. Comprehensive assessments of biocides used in hospitals and other healthcare settings and recent results on mode of action and the potential for microbial resistance are available (McDonnell & Russell 1999, Beumer et al. 2000, White & McDermott 2001).

Mode of Action of Biocides

Biocides have a long history of use as antiseptics and disinfectants, and play a key role in infection control and the prevention of nosocomial infections (McDonnell & Russell 1999). Despite their extensive use and long history, the mode of action of a number of biocides has not been clearly established (Denyer 1995). Biocides affect a number of different target sites in microorganisms (as shown in Table 1), which appears cumulatively to result in a loss of microbial viability.

In contrast to biocides, antibiotics, a class of naturally occurring or synthetic organic molecules, affect a specific target site in microorganisms resulting in bacteriostatic and bactericidal effects at therapeutic concentrations. Recent epidemiological surveys indicate an increase in the isolation of clinical strains of bacteria resistant to antibiotics (Walsh 2000). However, biocides inhibit a variety of microbial cellular processes (Denyer 1995, McDonnell & Russell 1999). The effect of biocides on multiple target sites in microorganisms is probably the principal reason for the lack of development of bacterial resistance to biocides. A summary of the differences between biocides and antibiotics is shown in Table 2. Several biocides have been utilized as oral care antiseptics for decades without any adverse microbiological reports (Slots 2000).

Clinical Studies Indicate ‘Real-Life Use’ of Antiplaque Biocides Does Not Result in Microbial Resistance amongst Oral Bacteria

The human mouth harbours a number of microorganisms that colonize a variety of surfaces (Socransky & Haffajee 1994) and are typically found as a biofilm (Gilbert et al. 1997). Many types of oral bacteria are commonly found adhering to tooth surfaces as dental plaque. The uncontrolled accumulation of oral bacteria in dental plaque has been associated with a number of clinical conditions, including caries, gingivitis and periodontal disease (Socransky & Haffajee 1992). Therefore, current practices of preventive dentistry are directed at reducing dental plaque in an effort to reduce the incidence of these conditions.

Table 1. Commonly used biocides in personal care products

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Mode of action</th>
<th>Typical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>Loss of structural organization, membrane damage, congealing of cytoplasm at high concentrations</td>
<td>Mouth-rinse, oral spray, dentifrice, hand disinfectant</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Membrane damage, release of cellular components</td>
<td>Dentifrice, hand soap, liquid soap</td>
</tr>
<tr>
<td>Cetylpyridinium chloride</td>
<td>General membrane damage</td>
<td>Mouth-rinse, hand disinfectant</td>
</tr>
<tr>
<td>Phenolic flavours</td>
<td>Protein denaturation, cell membrane damage</td>
<td>Mouth-rinse, lozenges, chewing gum</td>
</tr>
<tr>
<td>Metals (zinc, stannous)</td>
<td>Binding of -SH (thiol) groups</td>
<td>Dentifrice, mouth-rinse, chewing gum</td>
</tr>
<tr>
<td>Detergents (e.g. sodium dodecyl sulphate)</td>
<td>Structural/functional changes, initiation of autolysis</td>
<td>Foaming agent, detergent</td>
</tr>
<tr>
<td>Povidone iodide</td>
<td>Binding of thiol groups</td>
<td>Hand disinfectant, oral irrigant</td>
</tr>
</tbody>
</table>

Table 2. A comparison of the differences between biocides and antibiotics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Biocide</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Chemical agent</td>
<td>Natural or synthetic organic substance</td>
</tr>
<tr>
<td>Mode of action</td>
<td>Non-specific, such as membrane damage, protein denaturation and others, described in Table 1</td>
<td>Specific, such as inhibition of protein or DNA or cell wall synthesis</td>
</tr>
<tr>
<td>Spectrum of activity</td>
<td>Broad spectrum, effective against a range of microorganisms</td>
<td>Most often effective against a select group of microorganisms</td>
</tr>
<tr>
<td>Active components in formulation</td>
<td>Biocide and other ingredients including surfactants</td>
<td>Antibiotic only</td>
</tr>
<tr>
<td>In-use concentration</td>
<td>Substantially higher than MIC</td>
<td>At or slightly higher than MIC</td>
</tr>
<tr>
<td>Increase in MIC and antimicrobial efficacy</td>
<td>Correlation between MIC and efficacy is unclear</td>
<td>Correlates with therapeutic failure</td>
</tr>
<tr>
<td>Current use</td>
<td>Widespread use, e.g. in homes, in water treatment, to clean surfaces, in food</td>
<td>Used in human and veterinary medicine</td>
</tr>
<tr>
<td>Resistance</td>
<td>Not by single point mutation</td>
<td>By both single and multiple point mutations.</td>
</tr>
</tbody>
</table>
Table 3. Minimum inhibitory concentrations (MICs) of oral bacteria for commonly used biocides

<table>
<thead>
<tr>
<th>Agent</th>
<th>Effective against</th>
<th>MIC range of laboratory or fresh clinical isolates (µg/ml)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>0.2–6.2</td>
<td>Gaffar et al. (1990)</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Gram-positive and gram-negative bacteria</td>
<td>1–20</td>
<td>Walker (1988)</td>
</tr>
</tbody>
</table>

Table 4. Long-term clinical studies evaluating the microbiological safety of dentifrices containing antiplaque biocides

<table>
<thead>
<tr>
<th>Dentifrice tested</th>
<th>Investigators</th>
<th>No. of subjects</th>
<th>Study duration (months)</th>
<th>Development of opportunistic bacteria</th>
<th>Development of pathogenic bacteria</th>
<th>Duration sampled post-therapy for resistant bacteria</th>
<th>Development of resistant bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan/copolymer</td>
<td>Zambon et al. (1990)</td>
<td>81</td>
<td>7</td>
<td>No</td>
<td>No</td>
<td>6-week intervals for 6 months</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Bonta et al. (1992)</td>
<td>74</td>
<td>12</td>
<td>No</td>
<td>No</td>
<td>6-week intervals for 6 months</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Walker et al. (1994)</td>
<td>136</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zambon et al. (1995)</td>
<td>159</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>6-week intervals for 6 months</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Renvert &amp; Birkhed (1995)</td>
<td>114</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>6-week intervals for 6 months</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Fine et al. (1998)</td>
<td>68</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>12-week intervals for 6 months</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Rosling et al. (1997a)</td>
<td>40</td>
<td>36</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Triclosan/zinc citrate</td>
<td>Jones et al. (1988a)</td>
<td>26</td>
<td>7</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Stephen et al. (1990)</td>
<td>40</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Zinc citrate</td>
<td>Jones et al. (1988b)</td>
<td>2112</td>
<td>36</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Essential oil</td>
<td>Charles et al. (2000)</td>
<td>63</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Stannous fluoride</td>
<td>Weber et al. (1995)</td>
<td>120</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Grönoos et al. (1995)</td>
<td>34</td>
<td>12</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Maynard et al. (1993)</td>
<td>146</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Amine/stannous fluoride</td>
<td>Mengel et al. (1996)</td>
<td>150</td>
<td>9</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>

Clinical approaches such as mechanical removal of plaque with adequate oral hygiene procedures play an important role; however, poor patient compliance has reduced their usefulness (Wilson 1996, Löe 2000). Thus, a number of antibacterial agents, such as chlorhexidine, triclosan, essential oils, metal salts (e.g. zinc citrate and stannous fluoride) and extracts of plants, have been formulated in oral care products in efforts to inhibit dental plaque (Cummins & Creeth 1992, Marsh & Bradshaw 1995, Weber et al. 1995, Fine 1996, Cummins 1997, Gaffar et al. 1997, Eley 1999, Löe 2000, Pitten et al. 2000). Other antimicrobial agents such as povidone iodone, dilute sodium hypochlorite and chlorhexidine are routinely used for management of periodontitis (Slots & Jorgensen 2000).

In laboratory tests, antibacterial agents, including triclosan and chlorhexidine, demonstrate broad-spectrum activity against a variety of oral bacteria. Typically, the minimum inhibitory concentrations (MIC) of triclosan and chlorhexidine are in the range of 0.2–20 µg/mL, as summarized in Table 3.

The Council on Scientific Affairs of the American Dental Association has proposed guidelines for the acceptability of antibacterial agents in oral care formulations for the control of dental plaque. The guidelines state that the use of these agents should not result in the growth of pathogenic or opportunistic microorganisms or alter the composition of the oral flora (American Dental Association 1986). Based on these guidelines, a number of long-term clinical investigations have been conducted with both dentifrices and mouth-rinses formulated with a variety of antimicrobial agents to examine the effects of long-term use of these formulations on the oral flora. Furthermore, the oral flora has been monitored to examine changes in the proportions of bacteria and the development of bacterial resistance. Studies on both dentifrices and mouth-rinses formulated with extensively investigated antimicrobial agents are discussed below.

Dentifrices

Dentifrices are a common vehicle for routine patient-directed oral hygiene. A number of antiplaque biocides such as triclosan, metal salts, essential oils and chlorhexidine have been formulated in dentifrices to provide additional benefits such as reductions in plaque and gingivitis. Discussed in this section are the long-term effects of these antiplaque biocides on oral bacteria. A summary of these studies is presented in Table 4.

Triclosan, a broad-spectrum biocide with effects on many types of bacteria, has been extensively formulated in dentifrices. An interesting approach has been described to improve the delivery of triclosan on dental plaque following the addition of a copolymer of polyvinyl methyl ether (PVM) and maleic acid (MA) to dentifrices. Extensive clinical studies utilizing real-life conditions have been conducted to document a variety of benefits including reductions in plaque and gingivitis following use of the dentifrice with triclosan/copolymer.
Actinomyces triclosan/copolymer dentifrice. As representatives of supragingival plaque, Actinomyces spp., and Veillonella spp., were noted following use of the dentifrice for 6 months with the triclosan/copolymer dentifrice and after an additional 6 months post-use. A number of parameters, including the emergence of opportunistic bacteria, levels of putative periodontal pathogens and caries-associated oral microflora, in addition to the emergence of triclosan-resistant bacteria were evaluated in comparison with brushing with a placebo. No differences in the ratio of aerobic to anaerobic organisms or in the numbers of putative periodontal pathogens, including Aaactingobacillus actinomycetemcomitans, black-pigmented bacteroides (BBP) and Eikenella spp., were noted following use of the triclosan/copolymer dentifrice. As representatives of supragingival plaque, Actinomyces spp. and Veillonella spp., were isolated from patients using both the placebo and the triclosan/copolymer dentifrice. The MIC of these clinical isolates was determined at 3-month intervals for 12 months (including a 6-month period of post-therapy monitoring). At all sample times, there were no differences in the MIC of the isolates from the control and triclosan/copolymer groups. Finally, the study results indicated that opportunistic organisms comprised a relatively insignificant 1% of the total flora, with no significant changes noted for the duration of the study.

An additional exhaustive clinical study on microbiological safety following 6-month use of a placebo and the triclosan/copolymer dentifrice was reported (Zambon et al. 1995). In this study with 159 patients, there were no differences between the groups in the number of triclosan-resistant bacteria for the 6-month duration of the study. The supragingival plaque bacteria were characterized on 17 selective and non-selective bacteriological media and 90 microbiological parameters evaluated at several time points during the study. At the conclusion of the 6-month study, the supragingival plaque of subjects was assessed microbiologically for an additional 6 months post-therapy at 6-week intervals. These investigators reported no shifts in the presence or proportions of different bacteria in the supragingival plaque following use of the triclosan/copolymer dentifrice.

The results of the above studies showing a lack of triclosan resistance and no development of opportunistic flora are corroborated by the findings of earlier clinical studies (Zambon et al. 1990, Bonta et al. 1992, Walker et al. 1994, Renvert & Birkhed 1995). The studies compiled extensive microbiological profiles from over 300 subjects at various time points who brushed for up to 12 months either a placebo or the triclosan/copolymer dentifrice. An interesting result from these studies was the presence of a more health-associated flora reflective of immature plaque with higher proportions of Actinomyces spp. and streptococci amongst the triclosan/copolymer dentifrice.

Other clinical studies examined the effects of brushing with the triclosan/copolymer dentifrice for 3 years on the subgingival microbial flora of adults at risk of destructive periodontitis (Rosling et al. 1997a). Subjects using the triclosan/copolymer dentifrice demonstrated an overall decrease of all subgingival bacterial flora, with fewer subjects harbouring putative periodontal pathogens. No alterations were found in the microbial ecology of the subgingival regions including those bacterial species associated with health. Thus, clinical studies demonstrate that long-term use of the triclosan/copolymer does not alter the microbial populations in other regions of the mouth.

The efficacy of a dentifrice formulated with triclosan and zinc citrate on plaque and gingivitis has been described (van der Ouderaa & Cummins 1991, Cummins 1997). In an unreviewed 7-month brushing study, the use of the triclosan and zinc citrate dentifrice did not alter the composition of the plaque microflora or the proportion of bacteria comprising plaque (Jones et al. 1988a). The MIC to triclosan of clinical isolates of Streptococci and Actinomyces spp. that predominate in plaque showed no alterations, indicating no development of microbial resistance. These results were supported by the findings of an additional 6-month clinical study that examined the effects of continuous use of a dentifrice containing 0.5% zinc citrate and 0.2% triclosan with a 3-month post trial evaluation (Stephen et al. 1990). No shifts in oral flora or development of bacterial resistance to triclosan were reported. Similarly, a 3-year study with a large group of children brushing with a zinc citrate dentifrice did not demonstrate any changes in the oral flora (Jones et al. 1988b).

The results of a long-term (6-month) microbiological safety study with an essential oil-containing toothpaste were recently reported (Charles et al. 2000). Salivary and plaque bacterial isolates from the subjects at the start of the study and after brushing with an essential oil containing dentifrice for 3 and 6 months were collected for susceptibility tests. No alterations in the susceptibility of common oral bacteria to the essential oil dentifrice were reported at the various time points of the study. This clinical study also demonstrated no alterations in the normal oral flora of the subjects or development of opportunistic microorganisms over the course of the study.

The effects of 6 months of brushing with a dentifrice formulated with stannous fluoride on the oral microflora have been examined (Weber et al. 1995). As reported above for the triclosan/copolymer and triclosan/zinc citrate studies, no alterations in the oral microflora or opportunistic microorganisms were noted. The investigators also demonstrated that clinical isolates obtained over the course of the study remained susceptible to stannous fluoride.

Mengel et al. (1996) reported the effects of 9-month use of an amine stannous fluoride toothpaste and mouthwash on oral microflora. The supragingival plaque bacteria were morphologically characterized and indicated reductions in spirochaetes and curved rods.

Chlorhexidine (CHX) formulated in varnishes, gels and mouthwashes has been tested in clinical studies for its ef-
fects on mutans streptococci to prevent caries. A large number of studies have examined the effects of these treatments on the oral streptococci and have been previously reviewed (Emilson 1994). Discussed below are recent studies that have examined large numbers of bacterial strains following the use of CHX in gels or dentifrices. An interesting clinical study examined the susceptibility of 379 clinical isolates of mutans streptococci to CHX obtained from pregnant women over time (Grönnroos et al. 1995). Clinical isolates were obtained prior to child delivery and at various time points for 1 year. The test group of women brushed with a 0.3% CHX gel toothpaste for the first 10 days of each month during the course of the study. The sensitivities of all isolates from each subject collected at different time points were determined at the conclusion of the study to examine variations in CHX susceptibility within an individual over time. The results showed that intermittent use of the CHX gel did not result in the development of resistant bacteria for the duration of the test. Other clinical studies conducted recently and discussed below indicated that large numbers of oral streptococci isolated from patients prescribed CHX remain sensitive to CHX and commonly used antibiotics.

Clinical studies have also examined the effects of brushing for 6 months with a 1% CHX dentifrice on the oral microflora (Maynard et al. 1993). Plaque samples from 146 subjects were collected pre-brushing and after 6 and 24 weeks of brushing for microbiological analysis and determinations of the MIC to CHX. The results showed no alterations in the microflora, and no overgrowth by Candida spp. or other bacteria. Randomly collected plaque bacterial samples exhibited a slight increase in the MIC to CHX. These results were within the variations of the microbiological test procedures and were reported by these investigators as clinically insignificant with no development of bacterial resistance. Alterations in the sensitivity of oral bacteria to CHX were also reported in a previous study following a 1-year treatment with a 0.5% CHX gel (Emilson & Fornell 1976). This study, conducted with a small group of subjects, demonstrated the isolation of some oral streptococci with the ability to grow on plates containing up to 50 µg/mL of CHX. However, these effects declined post-trial with no alterations in the oral flora or overgrowth by opportunistic microorganisms reported, as has been reported for CHX rinses (see section on rinses below). Thus, taken together, results from a large number of clinical studies conducted in accordance with ADA guidelines (summarized in Table 4) indicate that the long-term use of antiplaque biocides in dentifrices has not resulted in the development of resistance or overgrowth by pathogenic or opportunistic bacteria.

Mouth-rinses

Mouth-rinses with antiplaque agents have a long history as an important adjunct for the oral care needs of patients. A variety of antimicrobial agents have been formulated in mouth-rinses and a number of long-term studies conducted to examine clinical efficacy. Mouth-rinses formulated with CHX are widely used to provide significant benefits in reducing gingivitis and plaque accumulation (for a review see Walker 1988, Mandel 1994). Other applications of CHX include use as a subgingival irrigant and as preprocedural rinses to control aerosols (Löe 2000, Slots & Jorgensen 2000). In clinical studies, the use of CHX rinse resulted in significant reductions of the subgingival plaque microbial flora. The effects on subgingival microflora of rinsing with a CHX mouth-rinse have been examined. A 6-month clinical study demonstrated a reduction in the number of oral bacteria with no overgrowth by Candida albicans or Escherichia coli (Newman et al. 1990). Although the side-effects of long-term CHX use include tooth staining, no emergence of opportunistic pathogens or stable shifts in the oral flora following extended use have been reported (Walker 1988, Löe 2000).

A number of studies have examined the ability to generate oral bacteria resistant to CHX. For example, Hennessey (1973) reported difficulties in isolating CHX-resistant mutants of oral streptococci and E. coli. However, Westergen & Emilson (1980), working with oral isolates of Streptococcus sanguis, reported the ability to generate stable CHX-resistant strains in the laboratory. The rates of spontaneous bacterial mutation in response to CHX appear to be lower than $10^{-5}$–$10^{-6}$. These resistant strains demonstrated an increase in MIC by 2–3 dilution steps. In a human clinical study conducted over 2 years, a slight increase in the MIC of the total salivary flora and oral streptococci during the course of the study was reported (Schiott et al. 1976). However, these alterations in MIC were transient and not seen 5 months after the completion of the trial, with no alterations of the oral microflora. Collectively, the results from a number of clinical studies have established the safety and efficacy of CHX without the development of resistant organisms (Mandel 1994, Eley 1999, Löe 2000, Pitten et al. 2000).

The efficacy of 2% CHX as a spray to successfully eradicate throat carriage of methicillin-resistant Staphylococcus aureus was reported from a preliminary study (Balfour et al. 1997). Other studies have demonstrated antiplaque efficacy following the use of a CHX chewing gum (Eley 1999). These reflect additional areas for the utilization of CHX.

The effects of 6-month use of an essential oil mouth-rinse on the bacterial composition of supragingival plaque were determined in two clinical studies (Minah et al. 1989, Walker 1988). No alterations in the bacterial composition of supragingival plaque or the emergence of opportunistic pathogens were reported. The bacteria remained susceptible to this essential oil during the course of these studies (Ross et al. 1989, Mandel 1994).

Clinical studies have examined the effects of 6-month use of mouth-rinse containing 0.1 or 0.2% delmopinol (Elworthy et al. 1995). Plaque samples were analysed at baseline and 12-week intervals for 36 weeks for shifts in bacterial populations and susceptibility to delmopinol over time. Results indicated no alterations in the microflora, including gram-negative bacteria, or in colonization by Candida spp. or other gram-negative aerobic bacteria. The susceptibility to delmopinol of plaque bacteria from subjects also showed no alterations during the study.

Klock et al. (1985) examined the effects of rinsing for 2 years with a mouth-rinse containing 0.4% stannous fluoride in comparison with a fluoride mouth-rinse on the numbers of Streptococcus mutans in caries-active subjects. They reported that twice-daily use of the stannous fluoride rinse resulted in significantly fewer sites with gingival bleeding, less gingivitis and decreased numbers of S. mutans after 1 year of use. A significant decrease in the num-
bers of *S. mutans* was evident after 2 years of use at the conclusion of the study.

In a clinical study with 102 subjects, the effects of extended (7-month) use of an amine fluoride and stannous fluoride rinse on the supragingival microflora were examined by dark-field microscopy (Zimmermann et al. 1993). The results showed that the use of the amine fluoride and stannous fluoride rinse resulted in a significant decrease in motile rods and spirochetes, with an increase in the proportion of cocci.

Walker (1988) reported the results of a 6-month clinical study that determined the effects of a PVP-I/H2O2 formulation on the subgingival microflora of 20 subjects. The use of PVP-I/H2O2 resulted in a significant decrease in the total number of microorganisms and anaerobic bacteria without producing any shifts in the composition of the oral microflora. No emergence of oral pathogens or opportunistic organisms was reported in this study. The long-term effects of a number of other antiplaque agents, for example extracts of sanguinarina, have been examined (reviewed by Eley 1999). However, these agents are not extensively used in oral care formulations, nor have their effects on oral microflora following long-term use been elucidated.

In summary, extended use of a number of antiplaque agents formulated in either dentifrices or mouth-rinses has been shown in clinical studies to provide the benefits of reductions in plaque and gingivitis, without resulting in microbial resistance or shifts in the oral microflora leading to colonization by pathogenic or opportunistic microorganisms.

**Effects of oral care formulations with antiplaque biocides on biofilms**

A variety of surfaces in nature, including the human body, are colonized by microorganisms that occur in the form of films called biofilms. These films are formed as a result of interbacterial interactions and coaggregation of a variety of microorganisms. A prime example of a biofilm is bacterial plaque on teeth and on prosthetic devices placed in the human body. These biofilms contain a spatially organized community of microorganisms attached to non-shedding surfaces such as teeth. In addition to bacteria, biofilms contain extracellular polymers secreted by the organism(s) and those derived from the environment (Costerton et al. 1999). As these biofilms contain unique bacterial phenotypes compared to organisms in culture media and present other barriers for the action of antimicrobials, higher levels of biocides (the concentration required is an order of magnitude higher than MIC) are required to affect biofilms (Gilbert et al. 1997).

Formulations designed to deliver biocides in the oral environment consist of surfactants (allowing penetration of biocide into the biofilm) and antinucleating agents that prevent conversion of soft biofilm to hard (calcification), in addition to the biocide. The effects of mouth-rinse formulations (containing CHX or other agents) were determined in clinical studies with a combination of fluorescent dyes that measure the vitality of bacteria in plaque (Bercx et al. 1990). The results indicated that use of CHX mouth-rinse resulted in reductions in the plaque index and the vitality of the plaque in the 21-day experimental gingivitis model. Recently, the effect of a single use of a mouth-rinse with essential oils in reducing supragingival plaque vitality at 30 min post-treatment has been examined in a short-term clinical study (Pan et al. 2000). The investigators utilized fluorescence dyes to quantify living and dead bacteria in plaque. The results indicated that, in comparison with a saline rinse, the essential oil rinse treatment produced a significantly higher number of dead bacteria in the plaque.

The formulation of dentifrices with triclosan and a copolymer of polyvinyl methyl ether (PVM) and maleic acid (MA) has been shown to improve the delivery of triclosan to dental plaque. Experimental evidence indicates that this combination enhances the effects of triclosan. For instance, the copolymer enhances the delivery of triclosan from dentifrices in the oral cavity, with significant levels of triclosan found in the dental plaque at 14 h post-brushing (Gaffar et al. 1997). Reductions in supragingival plaque and gingivitis following the use of the triclosan/copolymer dentifrice have been conclusively demonstrated in many extensive long-term clinical studies (Volpe et al. 1996).

Recent research efforts have explored other biological effects of triclosan delivered to dental plaque. For instance, in both laboratory and clinical studies, the bacterial vitality within the intact dental plaque biofilm has been measured by a bacterial viability index using a combination of fluorescent dyes that selectively stain the living or dead bacteria (Herles et al. 1994, Netuschil et al. 1998, Arweiler et al. 2001). The studies examined whether the combination of surfactant/triclosan/copolymer permitted the biocide to gain entry into the intact biofilm and affect the bacteria within the biofilm matrix. The results of clinical studies utilizing confocal laser scanning microscopy and other approaches confirmed a concomitant reduction in plaque bacterial vitality index and dental plaque index after use of the triclosan/copolymer dentifrice or a CHX mouth-rinse (Arweiler et al. 2001). Further, treatment of established dental plaque with the triclosan/copolymer dentifrice or a CHX mouth-rinse demonstrated significant reductions in the plaque bacterial vitality index for up to 24 h post-treatment. These results therefore indicate the ability of these antiplaque biocide treatments to affect mature dental plaque. Earlier laboratory studies demonstrated that treatment of a group of oral bacteria growing as a biofilm with a triclosan-containing formulation resulted in reductions in bacterial vitality within the biofilm (Herles et al. 1994). Recent biochemical investigations have examined the effects of triclosan on *Streptococcus sobrinus* and *Porphyromonas gingivalis* (Villalain et al. 2001). Triclosan is incorporated into phospholipid membranes and compromises the functions of the bacterial cell membranes. Preliminary results revealed that triclosan affects several membrane enzymes of *S. mutans* growing in a biofilm (Phan et al. 2001). In summary, studies on the effects of the triclosan/copolymer dentifrice and antiplaque biocide-containing mouth-rinses on the dental plaque biofilm and the microorganisms within the plaque have indicated that these formulations reduce the plaque index and plaque bacterial vitality.

The effects of antiplaque biocide formulations for supragingival use on other regions of the mouth, such as the subgingival microorganisms, are of interest. Clinical studies have examined the effects of using antimicrobial formulations, in some cases together with oral irrigators for delivery. The effects of mouth-rinses containing CHX, essential oils or PVP-I/H2O2 and the triclosan/copolymer dentifrice on subgingival flora have been examined. In a 6-month study with a CHX mouth-
Epidemiological surveys show no increase in antibiotic resistance in clinical isolates of oral bacteria from subjects using antiplaque biocide formulations

In epidemiological studies, the susceptibilities of 424 clinical isolates of *S. mutans* from healthy Finnish subjects, including those prescribed CHX treatments for dental conditions, were determined (Jarvinen et al. 1993). Approximately 70% of the subjects or their family members had been prescribed CHX. All isolates were sensitive to CHX and also to six commonly used antibiotics, namely amoxicillin, penicillin, cefuroxime, erythromycin, tetracycline and sulfamethoxazole-trimethoprim, with no development of resistance. These findings are supported by the results of another study which determined the CHX susceptibilities of 863 clinical isolates of *S. mutans* and 53 clinical isolates of *Streptococcus sobrinus* from 58 subjects following several doses of CHX treatments (Jarvinen et al. 1995), including a CHX gel and varnish, for 1 week. The salivary bacteria were sampled before treatment and at 4, 12 and 28 weeks post-treatment. All isolates of *S. mutans* and *S. sobrinus* remained susceptible to CHX for up to 28 weeks post-treatment. All clinical strains were also sensitive to commonly used antibiotics, including amoxicillin, penicillin, cefuroxime and tetracycline, with no development of antibiotic resistance reported.

In a recent report by these investigators, the susceptibilities of 839 clinical isolates of *S. mutans* from 209 adult patients were examined. These patients included those never exposed to amalgam fillings, those with fillings and those whose fillings had been removed. The isolates of *S. mutans* recovered from these patients were tested for susceptibility to CHX and the antibiotics tetracycline, penicillin and cefuroxime (Leistevuo et al. 2000). The results indicated that the susceptibility profiles of these strains to CHX and the antibiotics tetracycline, penicillin and cefuroxime were comparable to their previous results reported in 1993. Additionally, the investigators reported no relationships between dental amalgams and bacterial resistance.

In an unrelated study, the antibiotic susceptibilities of 110 salivary isolates of oral *Streptococcus* spp. from 112 adolescent children from France to CHX and a number of common antibiotics were determined (Lion et al. 1997). The investigators reported that all isolates were sensitive to CHX and several antibiotics. The MIC for CHX was comparable to those reported previously (Jarvinen et al. 1993, 1995). Although the study did not determine additional parameters, such as oral care products used, oral health of the subjects or prior antibiotic use, it is of significance that no resistance in the oral *Streptococcus* spp. to biocides or antibiotics was found.

Taken together, despite the extensive use of CHX for various applications in dentistry, the results of available clinical studies in different populations demonstrate that clinical isolates of common oral bacteria remain sensitive to CHX and several commonly used antibiotics.


topical disinfection

Topical disinfection is a well-known practice for infection control (McDonnell & Russell 1999). Hand disinfection in the dental clinic is vital to reduce the transfer of microorganisms and the risk of infection. The bacterial hand flora from 100 dental students and operating theatre staff were examined to determine the effects of repeated exposure to CHX-containing detergent scrub and the use of cetlypyridinium chloride (CPC) impregnated gloves on increasing microbial resistance (Millns et al. 1994). The study included first-year dental students who served as a control with little exposure to disinfectants. Dental students in the second, third and fourth years represented study populations with increasing exposure to disinfectants, with theatre staff serving as the group with maximum exposure. There were no significant differences in the carriage of staphylococci amongst these groups, indicating no alterations in the skin flora following extensive disinfectant use. The clinical isolates obtained from all groups were equally susceptible to both CHX and CPC in rate of kill tests at concentrations equivalent to the MIC for a control strain. Thus, the authors concluded that repeated use of hand disinfection by dental health personnel under real-life conditions did not result in the emergence of resistant bacteria.

Conclusions

In summary, the data derived from clinical studies indicate that long-term use of oral care formulations with antiplaque biocides does not result in microbial resistance. In extensive clinical tests with a variety of formulations incorporating various agents (chlorhexidine, triclosan, etc.) no alterations of the microbial flora of dental plaque were reported. Examination of clinical isolates of bacteria from patients using antiplaque biocides did not demonstrate resistance to the biocide used or to several commonly used antibiotics. Further, analysis of the skin microflora from dental professionals routinely using biocides for hand disinfection in dental clinics indicated no alterations in the composition of the microbial flora or resistance to biocides. The effects of oral care formulations with antiplaque
bacterielle Resistenz – Ein Übersichtsartikel


Résumé

Biocides anti-plaque et résistance bactérienne: Revue

La dentisterie moderne insiste sur l’importance du contrôle de plaque pour améliorer la santé orale. L’utilisation de composés de soins dentaires contenant des biocides anti-plaque joue un rôle crucial dans les contrôles de plaque réalisées par les patients eux-mêmes. L’efficacité anti-plaque de ces composés a été étudiée très largement lors d’études cliniques sur le long terme mises au point selon des critères acceptés. Les résultats de ces études démontrent que l’utilisation prolongée de ces composés contenant des biocides bien connus tels la chlorhexidine te le triclosan réduisait la plaque supra-gingivale et la gingivite. Cette revue critique résume les résultats microbiologiques des études cliniques sur les composés contenant des biocides anti-plaque. Les résultats d’un certain nombre d’études cliniques sur le long terme conduits dans des conditions de vie réelle indiquent qu’il n’y aurait aucune altération pour les bactéries trouvées dans la plaque dentaire et aucune émergence de résistance bactérienne. De plus, des prélèvements de plaque suite à l’utilisation prolongée de ces biocides ne révèle aucune augmentation de la microflore résistante. De grandes quantités de bactéries orales communes isolées de patients utilisant de la chlorhexidine ne montrent pas d’augmentation de la résistance bactérienne envers la chlorhexidine ou des antibiotiques communément utilisés. Les effets de ces composés contenant des biocides anti-plaque sur la plaquette dentaire qui est organisée naturellement en biofilm furent examinés. Ces composés contiennent des biocides, des surfactants des polymères et d’autres composants qui sont efficaces contre les biocides. En résumé, les résultats des études en condition réelle sur l’utilisation de ces composés ne montrent pas d’émergence de microflore résistante ou d’altérations de la microflore orale, alors que ces composés ont démontré qu’ils apportent un avantage pour la réduction de la plaque et de la gingivite.

References


Address:
Abdul Gaffar
Colgate-Palmolive Company
909 River Road
Piscataway
NJ 08855
USA