Selection for high-level resistance by chronic triclosan exposure is not universal

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Objectives: To investigate the effect of triclosan exposure on the antimicrobial susceptibilities of numerically important dental bacteria.

Methods: A gradient plate technique was used to expose Fusobacterium nucleatum, Lactobacillus rhamnosus, Neisseria subflava, Porphyromonas gingivalis, Actinomyces naeslundii, Prevotella nigrescens, Streptococcus oralis, Streptococcus sanguis and Veillonella dispar repeatedly to escalating, sublethal concentrations of triclosan. Escherichia coli ATCC 8739 was included as an organism showing the triclosan resistance development trait. MIC values towards chlorhexidine, metronidazole and tetracycline were determined before and after biocide exposure.

Results: N. subflava, Pr. nigrescens Po. gingivalis and E. coli were highly susceptible to triclosan (MIC range 0.1–3.9 mg/L), whereas the lactobacillus and S. mutans were less susceptible (MIC range 15.6–20.8 mg/L). Triclosan exposure resulted in a highly significant (∼400-fold) reduction in triclosan susceptibility (P < 0.01) for the positive control E. coli, although its MICs towards chlorhexidine, metronidazole and tetracycline were not significantly altered. Minor (∼two-fold) decreases in triclosan susceptibility (MIC) occurred for Pr. nigrescens and in S. sanguis and S. oralis (MBC). Mean changes in susceptibilities (MIC and MBC) of the oral species to chlorhexidine, metronidazole and tetracycline did not exceed two-fold, although chlorhexidine MBCs for S. sanguis were markedly, but transiently, increased.

Conclusions: These data fail to demonstrate biologically significant drug resistance in triclosan-exposed bacteria and suggest that markedly decreased triclosan susceptibility, although confirmed for E. coli, is not a universal phenomenon. Other bacteria possibly possess more susceptible targets than FabI that are highly conserved, which may govern triclosan activity.

Keywords: dental plaque, susceptibility, antibiotics

Introduction

The control of dental plaque, a complex microbial biofilm that accumulates naturally on tooth surfaces, is central to contemporary regimens for maintaining oral health.1 Mouthrinse and dentifrice formulations with antimicrobial agents serve as important adjuncts for the routine control of dental plaque.2 The agents commonly found in these formulations include chlorhexidine, triclosan, zinc salts and antimicrobial herbal extracts, with clinical studies demonstrating their antiplaque efficacy.3 For example, chlorhexidine is highly effective for the control of supragingival and subgingival bacterial communities,4,5 and the control of periodontal disease.6 An oral hygiene regimen with the triclosan/copolymer dentifrice significantly reduced pre-existing plaque and gingivitis above that accomplished by traditional fluoride-containing dentifrices.7 Independent clinical investigations demonstrate no adverse perturbations of the oral microflora, or changes in microbial susceptibility to antimicrobial agents after extended (6 months) use of this dentifrice.8 In addition, pharmacokinetic studies revealed no accumulation of triclosan in humans after exposure.9 As a group, formulations with antiplaque agents have a long history (20–50 years) of safe and efficacious oral use. Extended clinical studies demonstrated no association with the emergence of strains with reduced biocide susceptibility,10–13 while providing significant clinical benefits.

Recent laboratory studies have examined the possible sublethal effects of triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether)14 exposure on antimicrobial susceptibility, both towards triclosan and third-party antibiotics.15 In particular, molecular studies have
Triclosan exposure of dental plaque bacteria

demonstrated that triclosan interacts with an enoyl-acyl carrier protein (ACP) reductase (FabI) of the Gram-negative bacterium Escherichia coli. This enzyme, and its homologues, are essential in the fatty acid biosynthetic pathway of both Gram-positive and Gram-negative bacterial species. FabI mutants, resistant to triclosan, arise with moderately high frequency in E. coli cultures.

Whereas initial descriptions of triclosan resistance were related to a single FabI mutation, multiple mechanisms may be responsible for triclosan resistance. For instance, bacterial efflux pumps (RND family) that have a wide generic distribution can contribute significantly towards triclosan resistance. In E. coli, the AcrAB-TolC multi-drug efflux pump exhibits broad-spectrum action, targeting such compounds as antibiotics, quaternary ammonium compounds, salicylate and the disinfectant, pine oil.

Since triclosan is a substrate for most RND efflux pumps, exposure of bacterial cells to triclosan may select for regulatory mutants expressing these efflux pumps, thereby altering MICs to unrelated, third-party antimicrobials. There has been speculation that these observations might hold true for a range of bacteria, although to date, this has not been demonstrated.

If resistance development shown by E. coli is as widespread as the implicated ACP reductase, many bacteria may be considered as possible candidates for changes in drug susceptibility. In this study, we have simulated repeated (n = 10) environmental exposure of 10 selected oral bacteria, together with E. coli (as a control non-oral, bacterium, known to show the resistance development trait), to triclosan. The susceptibilities of the test bacteria towards a selection of antimicrobials commonly used in oral hygiene (the biocides triclosan and chlorhexidine), or in the treatment or oral infections (the biocides chlorhexidine, metronidazole and tetracycline), were determined, to give an indication of the possible implications of dental plaque exposed to this biocide.

Materials and methods

Chemicals

Unless otherwise stated, chemicals were obtained from Sigma (Dorset, UK). Formulated bacteriological media were purchased from Oxoid (Basingstoke, UK).

Bacteria

 Fusobacterium nucleatum ATCC 10953, Lactobacillus rhamnosus AC413, Neisseria subflava A1078, Porphyromonas gingivalis W50, Actinomyces naeslundii WVU627 and Prevotella nigrescens TS88 were obtained from Dr D. Bradshaw, BioSciences, Quest International, Ashford, UK. Streptococcus oralis NCTC 11427, Streptococcus sanguis NCTC 7863 and Streptococcus mutans NCTC 10832 were obtained from Dr J. Verran, Manchester Metropolitan University, Manchester, UK. E. coli ATCC 8739 was obtained from the American Type Culture Collection.

Maintenance of cultures

All bacteria (except E. coli and N. subflava) were maintained on Wilkins Chalgren anaerobe agar and broth in a Mark 3 Anaerobic Work Station (Don Whitley Scientific, Shipley UK) at 37°C (gas mix: 90% N2, 10% CO2 and 10% H2). E. coli and N. subflava were maintained on nutrient agar and broth and incubated aerobically at 37°C. E. coli was grown in anaerobic conditions for metronidazole-susceptibility testing. Bacteria were subcultured weekly by streaking onto the appropriate agar. All bacteria were stored at −70°C in nutrient broth containing glycerol (10%, v/v).

Exposure of test bacteria to triclosan

We used a Model CU spiral plater (Spiral Systems, Cincinnati, OH, USA) to create reproducible, radial concentration gradients of triclosan across agar plates. This system is a specialized dispenser, which distributes liquid samples in an Archimedes spiral onto the surface of a rotating agar plate from the centre to the edge, such that an ~1000-fold concentration gradient can be established, with potency decreasing from the centre to the edge. To facilitate repeated, sublethal exposure of the test bacteria to triclosan, a stock solution (1000 μg/mL) was prepared in 25% ethanol and filter sterilized (0.2 μm pore size; Millipore, UK) and stored at −70°C. Petri dishes (10 cm diameter) were filled with 27.5 ± 1.0 mL of Wilkins Chalgren or nutrient agar to produce a mean agar depth of ~3.5 mm. The plates were kept for 2 days at room temperature prior to use to ensure dryness of the agar surface. Stock solutions (50 μL) of the antimicrobial compounds were then deposited onto the agar surface using the variable cam of the spiral plater. Plates were then dried for up to 1 h at room temperature prior to radial deposition of inocula (20 μL) using a sterile inoculation loop. After further drying (1 h), plates were inverted and incubated anaerobiologically for up to 4 days at 37°C. For N. subflava, due to its high susceptibility, a triclosan stock solution of 10 μg/L was substituted for the 1000 μg/mL solution. After incubation, growth observed near to the endpoint, in the transition between growth and growth-inhibition area, was aseptically removed and homogenized in sterile, pre-reduced Wilkins Chalgren broth (1 mL). A portion (20 μL) was then transferred to the next triclosan gradient plate at the same concentration. This process was continued until 10 passages had been completed. Unexposed bacteria (P0), and those harvested after five (P5) and 10 (P10) triclosan passages, were stored at −70°C for subsequent MIC and MBC testing.

Determination of bacterial drug susceptibility (MIC)

Inocula for broth dilution endpoint determination of bacterial antimicrobial susceptibility were prepared as follows: single colonies of anaerobic test bacteria from uncontaminated agar plates were inoculated into sterile, anaerobic Wilkins Chalgren broth (10 mL) contained in 25 mL sterile plastic universal. Anaerobiosis was achieved by pre-reducing broth (placing them in the anaerobic cabinet immediately following autoclaving, at a temperature of at least 80°C). The aerobic bacteria N. subflava and E. coli were inoculated into nutrient broth and incubated in a standard aerobic incubator. Based on validation studies, the batch cultures of the test bacteria were incubated at 37°C for 24 h (±2 h) until they entered the early stationary phase. Cultures were then diluted to ~10−5 cfu/mL in sterile broth used as inocula in drug-susceptibility tests. Stock solutions (4 mg/mL) of chlorhexidine, metronidazole, and tetracycline were prepared in distilled water. Triclosan stock solutions were prepared in 25% ethanol. In order to reduce experimental variation, the total volume of each antimicrobial solution for these studies was prepared in advance, sterilized by filtration through single cellulose acetate filters (0.2 μm pore size, Millipore, UK), separated into aliquots (1.5 mL) and stored at −60°C. In all cases, controls were run for the 25% ethanol triclosan solvent. Testing was performed in 96-well microtitre plates (Becton Dickinson, Franklin Lakes, NJ, USA). Initial concentrations of the antimicrobial were 1000 μg/mL, except for testing of Po. gingivalis against tetracycline where the initial concentration was 100 μg/L. Diluted overnight culture (100 μL) was delivered to each test well. Antimicrobial solution (100 μL) was added to the first column of the test organism and mixed. Doubling dilutions were then carried out across the plate using a multi-channel pipette, changing the tips at each dilution step. The plates were then incubated for up to 48 h in either the anaerobic cabinet or
standard incubator at 37°C. The MICs were determined as the lowest concentration of antimicrobial at which growth did not occur. Growth was detected as turbidity (495 nm), relative to an uninoculated well using a microtitre plate reader (Anthos HTII; Anthos-Labtec Instruments, Salzburg, Austria). Each MIC determination was carried out in triplicate (in the same 96-well plate). Negative controls were performed with only sterile broth in each well and positive controls were performed with only overnight culture in the wells.

**Determination of lethal antimicrobial concentrations (MBC)**

After MIC testing of all 11 bacteria with chlorhexidine and triclosan at passage (P) 0, P5 and P10, MBC testing was carried out using the MIC 96-well plate. Aliquots of 10 μL taken from each well up to and including the MIC endpoint was transferred and spot-plated onto the appropriate agar and incubated overnight. MBCs were determined as the lowest concentration of biocide at which growth did not occur after 5 days of incubation.

**Statistical analyses.**

Replicated susceptibility data and the test bacteria before and after five and 10 triclosan passages were logarithmically transformed and then subjected to one-way analysis of variance (ANOVA).

**Results**

**Triclosan susceptibility**

Data in Table 1 show that *E. coli* underwent a highly significant, progressive increase in MIC and MBCs after five and 10 passages, with MIC values increasing by almost 400-fold at passage 10. Various minor (less than two-fold) changes in susceptibility were apparent for the test strains. *A. naeslundii* MICs transiently increased (P5), and MIC increases occurred for *P. nigrescens* and *S. sanguis*. MBCs increased for *S. oralis*, and the triclosan susceptibility of *L. rhamnosus* and *S. mutans* increased following biocide exposure. The most susceptible bacterium to triclosan was *N. subflava*, followed by the Gram-negative anaerobes *P. nigrescens*, *P. gingivalis* and *F. nucleatum*. The Gram-positive organisms *S. mutans* and *L. rhamnosus* were considerably less susceptible. The MIC:MBC ratios varied considerably among Gram-positive and Gram-negative organisms. The data shown in Table 1 demonstrate an apparent specificity of triclosan towards Gram-negative oral species, and that *E. coli* was the most responsive organism tested with respect to the changes in MIC value.

**Chlorhexidine susceptibility**

The MIC and MBC values for chlorhexidine (Table 2) after five and 10 triclosan exposures indicate that *L. rhamnosus* exhibited reduced susceptibility at P5 and a further reduction at P10. Susceptibilities to chlorhexidine after chronic triclosan exposure varied considerably within genera (streptococci) and within Gram-positive and Gram-negative groups. The bacterium most susceptible to chlorhexidine, with respect to the MIC data, was *A. naeslundii*, followed by the Gram-negative anaerobes *P. nigrescens*, *P. gingivalis* and *V. dispar*. *S. mutans* and *S. sanguis* were also highly susceptible. *F. nucleatum* and *L. rhamnosus* were considerably less susceptible. The MIC:MBC ratios for chlorhexidine were considerably higher for *S. mutans*, *P. nigrescens* and *A. naeslundii*.

**Metronidazole and tetracycline susceptibility**

The MIC values for metronidazole (Table 3) show that all the bacteria tested were poorly susceptible to this antibiotic, and that chronic exposure to triclosan had little effect upon the drug susceptibility. The bacteria least susceptible to metronidazole were *L. rhamnosus* and *F. nucleatum*. The bacterium most susceptible to metronidazole was the Gram-negative anaerobe *P. gingivalis*. The data demonstrate comparable MIC values among the streptococci tested in this study. Susceptibility to tetracycline also appeared to be largely unaffected (Table 3) by exposure to triclosan. This antibiotic was the most effective regarding MIC values, before and after triclosan exposure.

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**Table 1. Minimum inhibitory and minimum bactericidal concentrations of triclosan before and after chronic triclosan exposure**

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>MIC before</th>
<th>MIC P5</th>
<th>MIC P10</th>
<th>MBC before</th>
<th>MBC P5</th>
<th>MBC P10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 8729</td>
<td>0.1 (0.04)</td>
<td>15.3 (4.6)</td>
<td>39.1 (4)</td>
<td>0.2 (0.01)</td>
<td>19.5 (4)</td>
<td>39.1 (4)</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> WVU627</td>
<td>3.9</td>
<td>6.8 (1.7)</td>
<td>4.9 (1.7)</td>
<td>7.8</td>
<td>6.5 (1.8)</td>
<td>6.5 (1.8)</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em> ATCC 10953</td>
<td>7.8</td>
<td>3.9</td>
<td>9.8 (3.4)</td>
<td>7.8</td>
<td>7.8</td>
<td>6.5 (1.8)</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> AC413</td>
<td>15.6</td>
<td>13.7 (3.4)</td>
<td>6.8 (1.7)</td>
<td>15.6</td>
<td>15.6</td>
<td>7.8</td>
</tr>
<tr>
<td><em>Neisseria subflava</em> A1078</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
<td>0.1 (0.03)</td>
</tr>
<tr>
<td><em>Prevotella nigrescens</em> TS88</td>
<td>3.9</td>
<td>7.8</td>
<td>7.8</td>
<td>6.5 (1.8)</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em> W50</td>
<td>3.9</td>
<td>2.4 (0.8)</td>
<td>3.9</td>
<td>3.9</td>
<td>2.4 (0.8)</td>
<td>3.9</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> NCTC 10832</td>
<td>20.8 (7.4)</td>
<td>15.6</td>
<td>11.7 (3.9)</td>
<td>20.8 (7.4)</td>
<td>31.3</td>
<td>13 (3.7)</td>
</tr>
<tr>
<td><em>Streptococcus sanguis</em> NCTC 7863</td>
<td>2.6 (1.0)</td>
<td>3.9</td>
<td>3.9</td>
<td>5.2 (1.8)</td>
<td>26.1 (7.4)</td>
<td>7.8</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em> NCTC 11427</td>
<td>7.8</td>
<td>6.8 (1.7)</td>
<td>13 (3.7)</td>
<td>5.2 (1.8)</td>
<td>10.4 (3.7)</td>
<td>13 (3.7)</td>
</tr>
<tr>
<td><em>Veillonella dispar</em> NTCC 17745</td>
<td>3.9</td>
<td>3.4 (0.8)</td>
<td>4.9 (1.7)</td>
<td>7.8</td>
<td>7.8</td>
<td>5.2 (1.8)</td>
</tr>
</tbody>
</table>

Data were determined by broth dilution endpoint (doubling dilutions). ‘Before’ denotes bacteria previously unexposed to triclosan; P5 and P10, bacteria subjected to five and 10 triclosan passages, respectively. Units are mg/L. 

a Highly statistically significant (*P* < 0.01). 

b Statistically significant (*P* < 0.05). Data show means from triplicate experiments. Where data varied between replicates, standard deviations are given in parentheses.
passage. The bacteria most susceptible to tetracycline were the Gram-negative periodontal pathogens *Pr. nigrescens* and *Po. gingivalis*, and the Gram-positive cariogenic bacterium *S. mutans*. The bacteria least susceptible to tetracycline were *E. coli* and *V. dispar*.

**Discussion**

Following the demonstration that triclosan can select for tolerance in *E. coli*, the possible effect of triclosan upon triclosan susceptibility in various isolated bacteria and exposed populations has been subject to much discussion and scientific investigation. Despite the original observations of McMurry et al., few studies have unambiguously shown that bacteria other than *E. coli* can undergo such massive changes in triclosan susceptibility, although largely unsubstantiated claims persist.

Microbial communities within the oral cavity, arguably subject to considerable triclosan selection pressure, have not to date been associated with triclosan resistance. For example, Sullivan et al. showed that 14 days exposure to a triclosan-containing toothpaste did not affect the susceptibility of salivary streptococci towards triclosan and a range of antibiotics. Similarly, studies with dental plaque microcosms and longer-term human volunteer studies failed to show a clear association of triclosan exposure and biocide or antibiotic resistance. Since there have been few reports in the literature concerning the specificity and selectivity of triclosan against oral bacteria in pure culture, we have used a gradient plate technique to repeatedly expose a number of dental bacteria sublethally, together with a positive control (*E. coli*), to the biocide triclosan. This technique creates extremely selective conditions that are arguably more severe than could occur in situ.

In terms of triclosan specificity, the Gram-negative bacterium *N. subflava* was the most susceptible species, and the Gram-positive bacteria *S. mutans* and *L. rhamnosus* were the least susceptible. This is interesting, as one clinical application of triclosan is against

**Table 2.** Minimum inhibitory and minimum bactericidal concentrations of chlorhexidine before and after chronic triclosan exposure

<table>
<thead>
<tr>
<th></th>
<th>MIC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before P5 P10</td>
<td>before P5 P10</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8729</td>
<td>13.0 (3.7)</td>
<td>ND</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> WVU627</td>
<td>1.95 2.6 (0.9)</td>
<td>13.0 (3.7)</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em> ATCC 10953</td>
<td>15.6 7.8</td>
<td>20.8 (7.4)</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> AC413</td>
<td>10.4 (3.7) 15.6</td>
<td>13.0 (3.7) 15.6</td>
</tr>
<tr>
<td><em>Neisseria subflava</em> A1078</td>
<td>7.8 13.0 (3.7)</td>
<td>7.8 13.0 (3.7)</td>
</tr>
<tr>
<td><em>Prevotella nigrescens</em> T588</td>
<td>3.3 (0.9) 7.8</td>
<td>20.8 (7.4) 31.3</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em> W50</td>
<td>3.9 6.5 (1.8)</td>
<td>3.9 (2.7) 7.8</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> NCTC10832</td>
<td>3.9 6.5 (1.8)</td>
<td>83.3 (29.5) 125</td>
</tr>
<tr>
<td><em>Streptococcus sanguis</em> NCTC 7863</td>
<td>3.9 15.6 7.8</td>
<td>13.7 52.1 (14.7) 13.0 (3.7)</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em> NCTC 11427</td>
<td>7.8 13.0 15.6</td>
<td>7.8 13.0 15.6</td>
</tr>
<tr>
<td><em>Veillonella dispar</em> ATCC 17745</td>
<td>3.9 0.97 3.9</td>
<td>6.5 (1.8) 5.2 (1.8) 7.8</td>
</tr>
</tbody>
</table>

See legend to Table 1.

**Table 3.** Minimum inhibitory concentrations of metronidazole and tetracycline before and after chronic triclosan exposure

<table>
<thead>
<tr>
<th></th>
<th>Metronidazole</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before P5 P10</td>
<td>before P5 P10</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8729</td>
<td>250 125 125</td>
<td>15.5 13.0 (3.7) 10.4 (3.7)</td>
</tr>
<tr>
<td><em>Actinomyces naeslundii</em> WVU627</td>
<td>125 375 125</td>
<td>5.2 (1.8) 3.9 7.8</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em> ATCC 10953</td>
<td>250 250 500</td>
<td>3.9 3.9 2.9 (1.0)</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> AC413</td>
<td>500 500 500</td>
<td>5.2 7.8 3.9</td>
</tr>
<tr>
<td><em>Neisseria subflava</em> A1078</td>
<td>62.5 62.5 52.1 (14.7)</td>
<td>3.9 5.9 (2.0) 6.8 (1.7)</td>
</tr>
<tr>
<td><em>Prevotella nigrescens</em> T588</td>
<td>62.5 62.5 62.5</td>
<td>5.2 1.0 13.0 (4.0) 1.0</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em> W50</td>
<td>31.3 31.3 62.5</td>
<td>3.0 (1.0) 1.0 1.0</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> NCTC10832</td>
<td>62.5 62.5 62.5</td>
<td>5.2 1.0 1.0 2.0</td>
</tr>
<tr>
<td><em>Streptococcus sanguis</em> NCTC 7863</td>
<td>62.5 93. (31.3) 125</td>
<td>7.8 7.8 7.8</td>
</tr>
<tr>
<td><em>Streptococcus oralis</em> NCTC 11427</td>
<td>62.5 125 125</td>
<td>7.8 3.9 3.9</td>
</tr>
<tr>
<td><em>Veillonella dispar</em> ATCC 17745</td>
<td>78.1 13.7 31.3</td>
<td>31.3 31.3 27.4 (6.8)</td>
</tr>
</tbody>
</table>

See legend to Table 1.
methicillin-resistant *Staphylococcus aureus* and exploits its proposed anti-Gram-positive activity. These data therefore contradict some previous reports that triclosan has a low efficacy against Gram-negative species in general, although some *in vivo* oral studies have reported general reductions in plaque accumulation, with few detectable ecological shifts occurring.

Repeated exposure to triclosan (10 passages) caused a marked decrease in the triclosan susceptibility of the positive control, *E. coli*, reaching ∼400-fold in total (Table 1). Importantly, this is in agreement with previous investigations, which showed that that exposure of *E. coli* to triclosan either selected for a regulatory mutant expressing RND efflux pumps or caused a mutation in the FabI enzyme. None of the oral bacteria used in this study showed a biologically significant decrease in susceptibility to triclosan (MIC or MBC) after 10 passages. This suggests that triclosan could act more significantly upon other targets, such as membrane components in these bacteria, or that more sensitive highly conserved targets exist.

Interestingly, *E. coli* showed a marginal increase in susceptibility towards chlorhexidine following triclosan passage. This suggests that the increase in triclosan resistance seen in *E. coli* may result in fitness costs, or that the cells underwent short-term physiological damage during exposure to triclosan resulting in increased chlorhexidine susceptibility.

Tetracycline was the most potent antimicrobial used in this study for the majority of bacteria tested. The MIC values for metronidazole, however, were the highest obtained in this study for the bacteria tested, including the values for the obligately anaerobic species. This is surprising, as metronidazole is commonly employed to treat anaerobic dental infections and shows specific activity against obligately anaerobic species. The chronic exposure to triclosan of the 11 bacteria tested in this study had no apparent impact on their susceptibility towards metronidazole or tetracycline.

Much of the discussion concerning the hygienic use of triclosan has concerned possible changes in susceptibility towards chemically unrelated antimicrobials within triclosan-exposed bacteria. Recent environmental and microcosm studies have, however, failed to establish any clear link between triclosan use and antibiotic resistance. Our data for chlorhexidine, metronidazole and tetracycline demonstrate that repeated triclosan exposure does not result in major susceptibility changes among the bacteria used in this study. However, non-significant (two-fold or less) MIC decreases in *A. naeslundii*, *S. oralis* and *P. gingivalis* susceptibilities occurred towards metronidazole, and in *S. mutans* and *N. subflava* towards tetracycline. Overall, where minor changes did occur, they were probably attributable to short-term phenotypic adaptation, as opposed to mutation and selection. This data is in agreement with previous studies into dental bacteria in *in vitro* and *in situ* communities.

Triclosan has been successfully used in mouthrinses and dentifrice for over 20 years. To date, there have been no reports of a reduction in the effectiveness of this agent against dental bacteria. The increased use of triclosan in products used in the home, however, has dramatically increased the environmental exposure to triclosan, which has raised concern about possible cross-resistance to other antimicrobials. In this study, we have used repeated triclosan exposure to replicate previous studies whereby the triclosan susceptibility of *E. coli* was markedly decreased. Importantly, there was no evidence for concomitant selection of resistance to the other test agents, and the antimicrobial susceptibilities of the oral bacteria were not markedly changed.

In conclusion, we have confirmed that massive (−400-fold) decreases in triclosan susceptibility can be readily obtained by sublethally exposing *E. coli*. Common oral bacteria, subjected to identical selective conditions, showed few major changes in triclosan susceptibility. Where decreases in susceptibility did occur, they were over two orders of magnitude lower than occurred for *E. coli*. Similarly, triclosan exposure caused no net, significant decreases in susceptibility towards chlorhexidine, metronidazole and tetracycline in this study. This supports previous reports that suggest that the long-term use of triclosan products in the oral cavity does not select for triclosan-resistant populations.

**Acknowledgements**

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**Conflicts of interest**

Colgate-Palmolive markets a number of products that contain triclosan.

**References**