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ORIGINAL RESEARCH

In vitro Resistance Testing of *Porphyromonas gingivalis, Prevotella intermedia,* and *Tannerella forsythia* to Triclosan

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ABSTRACT

Aim: To determine the sensitivity of *Porphyromonas gingivalis, Prevotella intermedia*, and *Tannerella forsythia* to triclosan, and determine if these bacteria develop resistance to triclosan upon prolonged exposure.

Materials and methods: Susceptibility to triclosan was tested against three periodontal pathogens *P. gingivalis, P. intermedia*, and *T. forsythia. Escherichia coli* strains sensitive and resistant to triclosan were used as biological controls to confirm the efficacy of triclosan in the assays. Agar plates were prepared locally with vitamin K and hemin-supplemented medium.

Results: *Porphyromonas gingivalis* and *P. intermedia* did not grow on plates containing ≥ 2 µg/ml triclosan, while *T. forsythia* did not grow on ≥ 1.66 µg/ml. Colonies of *P. intermedia* resistant to triclosan developed after prolonged incubation at 2 µg/ml, but this resistance disappeared during subculture in the absence of triclosan.

Conclusion: No significant resistance to triclosan was detected for these species.

Clinical significance: Dental products containing triclosan can be beneficial in controlling periodontal disease.

Keywords: Laboratory research, *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia*, Triclosan susceptibility.

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Conflict of interest: None

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INTRODUCTION

Periodontal diseases are complex infections caused by several microorganisms, particularly the Gram-negative anaerobic rod species *Porphyromonas gingivalis, Prevotella intermedia*, and *Tannerella forsythia*.[1,2] Aggressive periodontal infections can be difficult to treat by debridement of subgingival plaque alone and adjunctive antibiotics are frequently used to supplement subgingival scaling and surgery.[3] Antibiotic therapy, however, can induce resistance in periodontal pathogens.[4,5] The bactericide triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) was developed as a synthetic biocide to suppress pathogenic bacteria responsible for several diseases. It has been incorporated into oral rinses and toothpastes to suppress species associated with periodontitis and dental caries.[6,7]

Resistance to triclosan has been observed for extraoral microbial species *Escherichia coli* and *Mycobacterium smegmatis*,[8] and resistant mutants, selected on agar plates containing triclosan, bear point mutations in an essential enzyme that is involved in the synthesis of fatty acids and thus membranes, and which is otherwise inhibited by triclosan.[9] In the present study, we measured the susceptibilities of *P. gingivalis, P. intermedia*, and *T. forsythia* to triclosan and looked for triclosan-resistant mutants by the plate assay.

MATERIALS AND METHODS

This study took place between Tufts University, School of Dental Medicine, in Boston, MA, USA, and The Forsythe Institute, in Cambridge, MA, USA. It was supported by NIH/NIDCR Grant DE-015847. Ethical approval from Tufts University, School of Dental Medicine, was obtained before the study was conducted.

Growth of reference strains of *P. gingivalis* ATCC BAA-1703 (FDC 381), *P. intermedia* ATCC 25611, and *T. forsythia* ATCC 43037 (FDC 338) was tested in the absence and presence of triclosan at concentrations ranging from 0.8 to 4 µg/ml. *Escherichia coli* strains AG100 (*E. coli* S) and *E. coli* AGT11 (*E. coli* R)[9] were used respectively, as triclosan-sensitive and triclosan-resistant biological controls to confirm the efficacy of triclosan in the assays. For triclosan sensitivity testing, all strains were grown anaerobically on a vitamin K and hemin-supplemented medium previously described,[10] but without blood unless noted. For the assays, cells harvested from agar, supplemented with 5% sheep blood, were suspended and spread over the surface of fresh 9 cm agar plates at two amounts 10-fold apart at around 10^7 cells/plate. Viable counts of inocula were measured on anaerobically serially diluted suspensions of inocula plated on blood-containing agar, to determine the numbers of viable cells actually plated. Growth in the presence or absence of triclosan, scored visually as the relative density of the lawn, was determined after 3 days for *E. coli*, and for *P. gingivalis, P. intermedia*, and *T. forsythia* after 10 days. Agar plates containing the oral pathogens were reincubated for three additional weeks to observe any further growth. Experiments were repeated in triplicate.

RESULTS

We found that the control-resistant *E. coli* (R) grew on the highest level of triclosan-containing agar plates (4 µg/ml), while no growth was observed for the sensitive *E. coli* (S) at the lowest level (0.8 µg/ml) (Table 1 and Fig. 1), as expected. After 10 days of anaerobic incubation, *P. gingivalis* was inhibited by 1.66 µg/ml triclosan for the low cell density and 2 µg/ml for the high cell density, while *P. intermedia* growth at both cell densities was inhibited by 2 µg/ml (Table 1 and Fig. 2). *Tannerella forsythia* growth was inhibited for the low cell density by 1.33 µg/ml triclosan and by 1.66 µg/ml for the high cell density (Table 1).

After additional incubation for 3 weeks, no growth was observed at inhibitory concentrations of triclosan for *P. gingivalis* or *T. forsythia*. For *P. intermedia,* however, new large (3 mm diameter) possibly triclosan-resistant mutant colonies appeared on the high-cell-density plates at 2 µg/ml triclosan (Table 1 and Fig. 3). These newly appearing colonies of *P. intermedia* were subcultured to blood agar plates to confirm species identification by cell and colony morphology. When these colonies of *P. intermedia* were subcultured on fresh plates with 2 µg/ml triclosan and on blood agar without triclosan, growth was seen as the emergence of single colonies on triclosan, and as a lawn on blood agar.

When cells from the blood agar plates lacking triclosan were then replated on media with 2 µg/ml triclosan, however, no growth was seen after three more weeks. In contrast, the cells from the triclosan plates continued

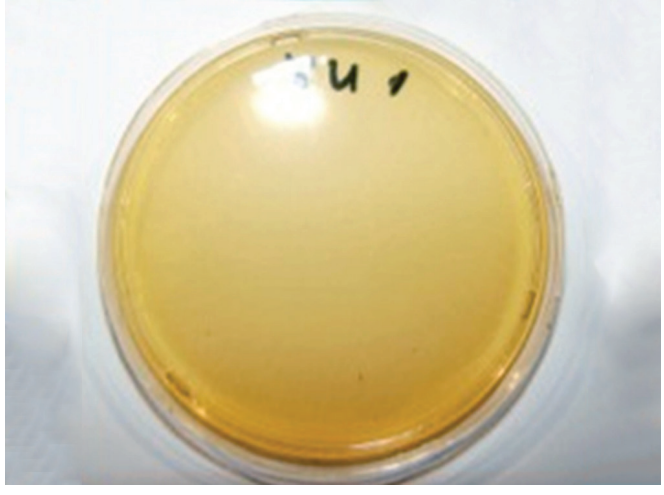

Fig. 1: No growth of the sensitive *E. coli* at the lowest triclosan level (0.8 µg/ml)

Table 1: Growth of *P. gingivalis*, *P. intermedia*, and *T. forsythia* on agar plates containing triclosan compared with growth of resistant (R) and sensitive (S) strains of *E. coli*

			*P. gingivalis**		*P. intermedia*†		*T. forsythia*‡	
Triclosan concentration (µg/ml)	*E. coli* R	*E. coli* S	*High*	*Low*	*High*	*Low*	*High*	*Low*
0	+	−	+	+	+	+	+	+
0.80	+	−	+	+	+	+	+	+
1.00	+	−	+	+	+	+	+	+
1.33	+	−	+	+	+	+	+	−
1.66	+	−	+	−	+	+	−	−
2	+	−	−	−	−§	−	−	−
3	+	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−

+: Growth; −: No growth; **P. gingivalis:* High concentration 8.4×10^7 cells, low concentration 8.4×10^6 cells; †*P. intermedia*: High concentration 3.3×10^7 cells, low concentration 3.3×10^6 cells; ‡*T. forsythia*: High concentration 4.1×10^7 cells, low concentration 4.1×10^6 cells; §*P. intermedia:* 10 ± 2 colonies grew after three additional weeks anaerobic incubation

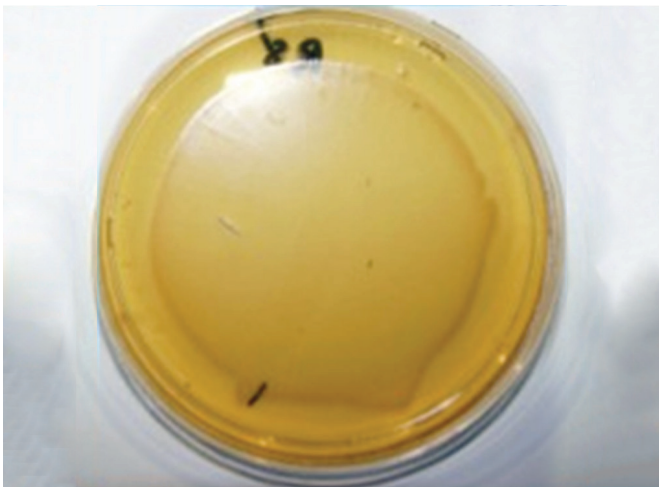

Fig. 2: Lawn growth of *P. gingivalis* at the lowest triclosan level (0.8 µg/ml)

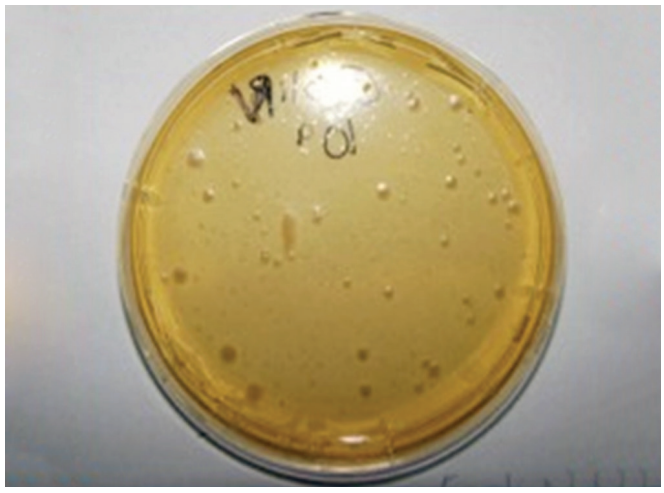

Fig. 3: Isolated colonies of *P. intermedia* developed at 2 µg/ml triclosan after prolonged incubation

growing every time they were replated on media with 2 µg/ml triclosan. Therefore, the resistance of the population of *P. intermedia* to triclosan was perpetuated only by continuous selection on triclosan, and so was unstable.

DISCUSSION

Porphyromonas gingivalis is generally sensitive to antibiotics and biocides. A mutation-causing gene has been found in this species, although resistance has not yet been detected.[5,11] In the current study, *P. gingivalis* demonstrated sensitivity at 2 µg/ml triclosan and did not show resistance *in vitro* with prolonged exposure to triclosan. This is consistent with previous *in vivo* studies in which 3-, 6-, and 12-month exposure to 0.3% (3000 µg/ml) triclosan showed growth attenuation and bacterial count decrease.[12]

Prevotella intermedia did not grow in 10 days at 2 µg/ml triclosan, again consistent with previous *in vivo* studies.[12] After even longer exposure to the 2 µg/ml triclosan, however, several new colonies of *P. intermedia* were observed, suggesting development of triclosan-resistant colonies. Nevertheless, the inability of this population to grow on 2 µg/ml triclosan following subculture to medium without triclosan suggests the enhanced resistance was not stable.

Tannerella forsythia rarely develops resistance to antibiotics,[13] and in this study, the test strain was sensitive to triclosan at 1.66 µg/ml, and did not show resistance after prolonged incubation.

CONCLUSION

We conclude that *P. gingivalis, T. forsythia,* and *P. intermedia* are quite sensitive to triclosan (inhibited by 2 µg/ml). This has not previously been reported for *T. forsythia*. An additional level of resistance for *P. intermedia* was observed as colonies emerging on 2 µg/ml triclosan with prolonged incubation. This resistance, however, proved to be unstable since it was not being maintained in the absence of the drug. Overall, the sensitivity of these subgingival species to triclosan is consistent with clinical observations of suppression of these species after oral application of triclosan-containing dentifrices.[7,12,14,15]

CLINICAL SIGNIFICANCE

Dental products containing triclosan can be beneficial in controlling the progression of periodontal disease. The concentration of triclosan in most triclosan-containing toothpastes today is 0.3%, which is equal to 300 µg/ml. This concentration is above the minimum inhibitory concentration, which in addition to the low resistance levels of the species tested makes triclosan safe to use and unlikely to induce resistance.

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