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Triclosan antimicrobial activity against dental-caries-related bacteria

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Triclosan (TCS) is a chlorinated diphenyl ether and a possible active agent against microorganisms. Due to its probability of reducing dental plaque accumulation, TCS can be added as a substance for oral hygiene. **Aim:** To evaluate the efficacy and antimicrobial capacity of TCS against *Pseudomonas aeruginosa* and *Streptococcus mutans*. **Methods:** This work evaluates the percentage of bacteria inhibition of *P. aeruginosa* (ATCC 27853) and *S. mutans* (ATCC 25175). TCS concentrations between 2 and 128 $\mu g.mL^{-1}$ were tested. **Results:** An inhibitory potential of TCS was found against *S. mutans*. No percentage of inhibition was detected against *P. aeruginosa* (technical and biological triplicate). **Conclusion:** TCS, an antimicrobial agent used in dentifrices, can reduce *S. mutans* levels therefore these dentifrices should be indicated for patients with a high risk of caries. However, further study is needed, including antimicrobial analyses against other microbial conditions.

Keywords: Dental caries. Triclosan. *Streptococcus mutans*.

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Introduction

Tooth decay happens as a consequence of enamel and dentin tissue degradation, resulting from bacteria-produced acids. Its consequences may include pulp inflammation, pain, infection, edema, and tooth loss[1]. Different bacterial species cause dental caries, and many bacterial strains were already characterized as an etiological factor[1]. *Streptococcus mutans* is described as one of the main etiological factors of dental caries[2]. This microorganism is capable of colonizing the oral cavity and forming bacterial biofilm[3]. In addition to *S. mutans*, several other bacteria are also present in dental biofilm. Studies demonstrate *P. aeruginosa* in saliva, and the subgingival and supragingival biofilm of subjects with chronic periodontal infection[4,5]. Also, *P. aeruginosa* is related to the failure of periodontal treatment[6], and the development of aggressive periodontitis[6].

Prevention of dental caries is directly related to biofilm remotion by flossing and brushing teeth[6]. In addition to the mechanical removal of dental biofilm, chemical agents are also good coadjuvants for oral health care promotion[6]. Fluoride, the most used substance to prevent tooth decay, is found in toothpaste and water[6]. Chlorhexidine gluconate (CHX) is found in mouthwashes and is the most indicated antimicrobial for patients with periodontal diseases[7]. In addition to these substances, other agents are already being used to increase the preventive effect of toothpaste.

Triclosan (TCS) is a chlorinated diphenyl ether or bisphenol of broad-spectrum against gram-positive and negative bacteria and fungi and is characterized as a non-ionic molecule[8]. Evidence indicates that TCS has antimicrobial capacity against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* besides having anti-inflammatory properties that reduce bacterial biofilm[9] and contribute to decreased bacterial load and a reduction in pathogenicity[10,11]. In addition, a study has demonstrated that TCS has an anti-inflammatory action, a long-lasting effect, and high substantivity, resulting in an active agent to reduce dental plaque accumulation[12]. Due to these properties, this antimicrobial is part of the composition of some toothpaste and mouthwashes[13]. Furthermore, the study suggests TCS could be incorporated as a component of glass ionomer cement.

In this context, TCS has been employed as an adjunct to fluoride. A study indicated that a dentifrice containing 0.3% of TCS was highly effective in preventing and enhancing demineralization compared to a positive control sodium fluoride dentifrice[14].

Therefore, it is essential to know the antimicrobial capacity of TCS against each oral bacterium, contributing to the correct indication of oral hygiene agents containing TCS. Our study hypothesized that TCS has an antimicrobial potential against *P. aeruginosa* and *S. mutans*, and this analysis may help indicate oral hygiene agents containing TCS for specific conditions. Thus, further studies should be performed to assess TCS activity on other microorganisms related to oral diseases.

Materials and Methods

Triclosan and ampicillin preparation

TCS (Via Magistral, DF, Brazil) was dissolved in 20% absolute ethanol and 80% sterile distilled water and used at different concentrations (serial dilutions from 2 to 128 $\mu g.mL^{-1}$). Gentamicin and chloramphenicol (Sigma Aldrich, MA, USA) were diluted in sterile distilled water and Brain Heart Infusion (BHI) broth (Thermo Fisher, MA, USA) in 10 $mg.mL^{-1}$[15].

Microorganisms' preparation

P. aeruginosa (ATCC27853) and *S. mutans* (ATCC 25175) were cultured in a Petri dish containing Muller Hinton (MH) agar (Sigma Aldrich, MA, USA) for *P. aeruginosa* and BHI agar (KASVI, USA) for *S. mutans*. The pre-inoculum preparation of each bacterium was carried out in a flow chamber, where three colonies of bacteria from each microorganism (biological replication) were selected and inoculated in 5 mL of MH broth and BHI broth. This culture was maintained under shaker conditions (200 rpm) at 37 °C, overnight. Inoculum of bacteria was obtained through 100 µL of the pre-inoculum of each bacteria and added to 4.9 mL of MH and BHI under agitation (200 rpm) at 37 ºC, for 1 hour. Optical density (O.D.) were performed until reaching 0.3 (*P. aeruginosa*) and 0.25 (*S. mutans*) at an absorbance (ABS) of 600 nm. In this ABS, $5.02x10^{11}$ CFU of *P. aeruginosa* and $1x10^{5}$ CFU of *S. mutans* were being considered. Technical and biological replicates were performed in a 96-well plate (TPP, USA)[15].

Triclosan's antimicrobial capacity

TCS (0.004 g) was weighed in Eppendorf (1.5 mL). TCS stock was obtained by diluting 200 $\mu g.mL^{-1}$ of absolute alcohol and 800 $\mu g.mL^{-1}$ of sterile distilled water. The antibiotic controls for each bacterium were diluted according to these antibiotics' concentrations in their respective media. All samples were separated in each group: 1) medium, 2) medium and alcohol, 3) medium and bacteria, 4) bacteria and antibiotics, 5) bacteria and alcohol, and 6) bacteria and different dilutions of Triclosan (128 to 2 $\mu g.mL^{-1}$). Soon after, plates were stored in an incubator at 37 ºC for 18 hours. The plates were read with an absorbance of 600 nm. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were obtained following the standards of a previous protocol. Percentage of bacteria inhibition was calculated from the absorbances of microdilution [15].

Statistical analysis

Technical and biological replicates were performed for all analyses. Mean of absorbances were calculated on Excel (Microsoft Software, San Diego, CA, USA). and antimicrobial analyses were determined by comparing samples to the controls referring to 100% and 0% of microbial growth. The graphics were made in Graphpad Prism (GraphPad Software, San Diego, CA, USA).

Results

Gentamicin MIC against *P. aeruginosa* was 10 µg.mL^{-1} while chloramphenicol MIC was also 10 µg.mL^{-1} against *S. mutans* (Table 1).

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in µg/mL^{-1} of Triclosan and control (gentamicin and *chloramphenicol)* against *Pseudomonas aeruginosa* and *Streptococcus mutans*. ND: non detected until 128 µg.mL^{-1}.

Microorganism	TCS		Control	
	MIC	MBC	MIC	MBC
S. mutans	ND	ND	10 µg.mL^{-1}	10 µg.mL^{-1}
P. aeruginosa	ND	ND	10 µg.mL^{-1}	10 µg.mL^{-1}

None of the TCS tested concentrations inhibited *S. mutans* and *P. aeruginosa* growth. Also, no TCS percentage of inhibition was detected against *P. aeruginosa* in technical and biological triplicates. TCS showed around 80% of inhibitory activity at 128 to 8 µg.mL^{-1} against *S. mutans* (Figure 1).

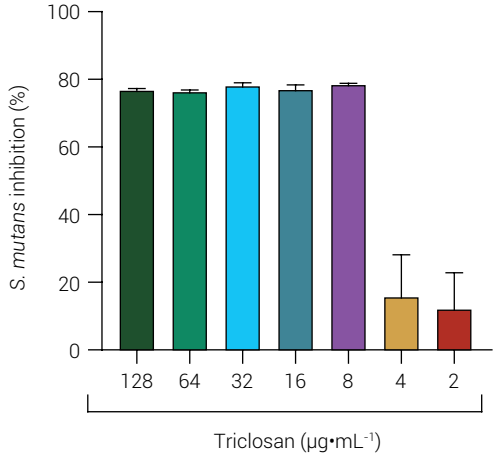

Figure 1. Percentage of *S. mutans* inhibition by various concentrations of Triclosan in µg/mL $^{-1}$.

Discussion

The TCS inhibitory results against the bacteria represented in this work are consistent with the data already reported in the literature. *S. mutans* is one of the primary causative caries, so it is essential to assess how this bacterium is affected by TCS[12]. Possibly, TCS is a multi-target inhibitor for *S. mutans*, which lack a triclosan-sensitive Fabi enoyl-ACP reductase, and that inhibition of glycolysis in dental plaque biofilms, in which TCS is retained after initial or repeated exposure, would reduce cariogenicity[16]. Besides, other studies report the relationship of TCS and *S. mutans*, where possibly the gene called FabK, an isoenzyme present in the bacterial cell membrane, may be the target of the TCS[17,18].

P. aeruginosa has already been reported to be highly resistant to TCS and most conventional antibiotics, such as carbapenems[19]. In the present study, TCS was tested at concentrations from 2 to 128 μg.mL^{-1}. However, none of these concentrations was able to inhibit the growth of *P. aeruginosa*. Even though the TCS has a protein carrying enoyl acyl reductase of the FabI type, an isoenzyme present in the bacterial cell membrane, such protein is considered a target of Triclosan[20]. However, it is believed that the FabV type enoyl acyl reductase carrier protein, which is also an isoenzyme present in the cell membrane of *P. aeruginosa*, confers resistance to TCS[20].

Thus, TCS can possibly be indicated for patients at high risk for caries due to its antimicrobial action against *S. mutans* observed in this study and its effectiveness as an adjuvant to fluoride and remineralization capacity already reported[14]. Although studies observed a decrease in dental plaque using toothpaste with triclosan[9,11,12], no study has compared the caries incidence of patients using TCS with other agents. Thus, further study is needed to confirm this indication.

Despite the resistance of *P. aeruginosa* to TCS, other studies demonstrate the indication of this antimicrobial in cases of gingivitis due to the antimicrobial action against *A. actinomycetemcomitans* and *P. gingivalis*[8]. Another study has already reported a decrease in periodontitis markers, such as bleeding on probing and probing depth, in children from parents with aggressive periodontitis who used toothpaste with triclosan[21]. Even though CHX is the most used antimicrobial for periodontal diseases, TCS is more compatible with typical toothpaste ingredients, unlike CHX which is mostly found in mouthwashes[7,22]. Also, CHX showed some disadvantages: staining the enamel surface[21] and a higher cost compared to TCS. Therefore, further study is needed, including testing different species of microorganisms related to periodontitis and gingivitis.

Our results reinforce the use of TCS as part of the composition of toothpaste, mouthwashes, and even dental materials such as glass ionomer cement, especially for patients with a high risk of caries[21]. Further study is needed to make TCS more present in these products.

In conclusion, it is estimated that the use of TCS against *P. aeruginosa* may be unfeasible due to its resistance, but other concentrations could be tested due to the clinical results already reported. However, this agent has an inhibitory potential against *S. mutans*, one of the main bacteria related to caries. Thus, these results suggest that it is highly recommended to use TCS as an oral hygiene agent in toothpaste and mouthwashes, as well as in other materials such as glass ionomer, due to its antimicrobial capacity. Also, it is possible to indicate toothpaste and mouthwashes with TCS for patients at high risk of caries. However, further work is needed to test the TCS in other microbial conditions and applications in these products.

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Author Contribution

Jade Ormondes de Farias: Roles/Writing - original draft, Writing - review & editing. Jamilca de Almeida do Espírito Santo: Methodology, Investigation, Data Curation. Ingrid Aquino Amorim: Methodology, Investigation, Data Curation. Taia Maria Berto Rezende: Conceptualization Resources, Funding acquisition, Supervision, Writing - review & editing. All authors actively participated in the manuscript's findings and have revised and approved the final version of the manuscript.

Data availability

Datasets related to this article will be available upon request to the corresponding author.

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