Virtual Screening to Identify the Protein Network Interactions of Triclosan with *Streptococcus mutans* and *Enterococcus faecalis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Triclosan is considered to be an important ingredient in toothpastes and mouth rinses. Several studies have reported contradictory results regarding the antimicrobial effect of triclosan. Hence, the present in silico study intends to identify the potential targets of triclosan in two common dental pathogens *Streptococcus mutans* and *Enterococcus faecalis*.

Aim: To identify the protein network interactions of triclosan in *Streptococcus mutans* and *Enterococcus faecalis* by virtual screening method.

Materials and Methods: The STITCH v5.0 database was initially used for identifying drug-protein interactions followed by VICMPred and VirulentPred which was employed to identify functional class of the proteins and its virulence property. Finally, BepiPred v1.0 Linear Epitope Prediction tool was used to identify the potential epitopes of the virulent proteins.

Results: Triclosan was found to interact with crucial proteins in *S. mutans* and *E. faecalis* which could contribute to severe forms of periodontitis and endodontic diseases.

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Conclusion: Taken together, the present study provides the preliminary data on the potential targets of triclosan in common dental pathogens. Further experimental validation is warranted to provide concrete evidence on the molecular targets of dental pathogens.

Keywords: Triclosan; Streptococcus mutans; Enterococcus faecalis; Periodontitis; antimicrobial agent; novel compounds.

1. INTRODUCTION

Triclosan (TCS), an antimicrobial agent, is present in products such as toothpaste, soaps, detergents, toys, and surgical cleansing solutions [1]. It is a common ingredient in soaps, shampoos, deodorants, toothpastes, mouthwashes, cleaning supplies, and pesticides, and part of consumer products like kitchen utensils, toys, bedding, socks, and trash bags etc [2]. Antimicrobial ingredients have long been used in various products to slow down or inhibit the growth of bacteria, fungi [3]. In hospital settings, 2% triclosan has been used in surgical units for the decolonization of a patient's skin carrying methicillin-resistant Staphylococcus aureus (MRSA)[4,5]. A study using commercially available toothpaste containing triclosan indicated a significant reduction in gingivitis, bleeding, and plaque[6] other systematic analysis by Cochrane group suggested that reduction in gingivitis, bleeding, and plaque may be statistically significant under in vitro conditions, but the same could not be replicated in a clinical setting. In other words, triclosan might not attain clinical significance. Our team has extensive knowledge and research experience that has translate into high quality publications[7–11]. Such contradictory results have posed queries about the use of triclosan as an effective antimicrobial agent. Hence, the present study was intended to identify the potential molecular targets of triclosan in common dental pathogens such as S.mutans and E.faecalis. Computational techniques are widely employed to screen for drug molecules and their targets in microbes and host. Several studies have been initiated by the authors previously to deduce the potential targets of synthetic [12] and phytocompounds [13], [14] against red complexes and other common dental pathogens. Virtual screening methods have been used to reduce cost and time. The prediction tools would provide preliminary information about the molecular targets of the drug in the microbial pathogens, which will be of great use to the researchers to identify drug molecules that would best suit their needs and be less toxic to the hosts.

2. MATERIALS AND METHODS

The present observational study aims to screen for those proteins of Streptococcus mutans and Enterococcus faecalis interacting with Triclosan. The protein-drug interaction of bacteria was analyzed using STITCH v.5 pipelines [15] and the functional class and virulence property of the interacting proteins was detected using VICMpred and VirulentPred softwares.

2.1 Prediction of Drug-Protein Interactions

The STITCH database helps in providing an exhaustive platform for known and predicted interactions between chemicals and proteins. The interactions could be direct or physical and indirect or functional associations.

2.2 Identification of Virulent Protein and Functional Class

VICMpred [16] and VirulentPred [17] software were used for the identification of virulence factors targeted by Triclosan among the Streptococcus mutans and Enterococcus faecalis. These tools employed a support vector machine (SVM)-based five-fold cross-validation process to validate results. Virulence factors were screened based on amino acid composition using the VirulentPred tool which classified them into two groups, that is, virulent and avirulent. VICMpred groups proteins into four major classes, namely, proteins involved in cellular processes, metabolism, information storage, and virulence.

2.3 Prediction of Epitopes

The BepiPred v1.0 Linear Epitope Prediction tool predicts B-cell epitopes from a protein sequence, using a Random Forest regression algorithm with trained five fold cross-validation approach was used to discriminate between epitopes and non-epitope amino acid residues from crystal structures. The residues with scores above the threshold (>0.5) are predicted to be part of an epitope and colored in yellow on the graph [18, 19].
Table 1. Proteins of *Streptococcus mutans* and *Enterococcus faecalis* interacting with triclosan

| Organism          | Identifier | Proteins which interacts with triclosan | VICMPred Functional Class | Virulent Pred | Virulent Pred Score |
|-------------------|------------|----------------------------------------|---------------------------|---------------|---------------------|
| *Streptococcus mutans* | SMU_38c    | Transcriptional regulator               | Information and Storage   | Virulent      | 0.7941              |
|                   | SMU_236c   | Transcriptional regulator               | Metabolism                | Virulent      | 1.0358              |
|                   | SMU_514    | Transcriptional regulator               | Cellular process          | Virulent      | 0.7518              |
|                   | SMU_346    | NADH dehydrogenase                      | Cellular process          | Non-virulent  | -1.040              |
|                   | SMU_1602   | NAD(P)H-flavin oxidoreductase            | Cellular process          | Non-virulent  | -1.032              |
|                   | SMU_439    | Transcriptional regulator               | Cellular process          | Virulent      | 1.0136              |
|                   | SMU_2134   | Transcriptional regulator               | Cellular process          | Virulent      | 1.0232              |
|                   | SMU_1240c  | Nitroreductase                          | Cellular process          | Non-virulent  | -1.043              |
|                   | SMU_1343c  | Polyketide synthase                     | Virulent                  | 0.8437        |
|                   | SMU_1336   | Conserved hypothetical protein PksD     | Virulence factors         | Virulent      | 1.0032              |
| *Enterococcus faecalis* | EF_1773    | 3-ketoacyl-ACP reductase                 | Cellular process          | Virulent      | 0.7671              |
|                   | EF_1690    | Short chain dehydrogenase/reductase     | Family oxidoreductase     | Non-Virulent  | .220                |
|                   | EF_0404    | Nitroreductase                          | Metabolism Molecule       | Non-Virulent  | -0.998              |
|                   | EF_3059    | TetR family transcriptional regulator   | Metabolism Molecule       | Virulent      | 0.8280              |
|                   | EF_2203    | TetR family transcriptional regulator   | Cellular Process          | Non-Virulent  | -1.051              |
|                   | EF_1181    | Nitroreductase                          | Metabolism Molecule       | Non-Virulent  | -0.613              |
|                   | EF_0648    | Nitroreductase                          | Metabolism Molecule       | Non-Virulent  | -0.990              |
|                   | EF_1326    | TetR family transcriptional regulator   | Metabolism Molecule       | Virulent      | 0.8759              |
|                   | EF_2171    | Epimerase/dehydratase                   | Metabolism Molecule       | Virulent      | 1.0538              |
|                   | EF_0282    | Enoyl-ACP reductase                     | Metabolism Molecule       | Virulent      | 0.6870              |
Fig. 1. Protein interaction network of (a) *Streptococcus mutans* and (b) *Enterococcus faecalis*

Fig. 2. Predicted epitopes on virulent factor conserved hypothetical protein PksD [SMU_1336] identified using computational tools

Chart 1. The list of predicted peptide epitopes on the virulent proteins identified
3. RESULTS AND DISCUSSION

Stitch software was used to classify protein interactions between *Streptococcus mutans* and *Enterococcus faecalis* against triclosan (Fig. 1). Furthermore, each of the proteins interacting with the drug was tested for their virulence properties using VirulentPred and functional properties via VICMpred. The scores provided by these algorithms were verified based on their amino acid sequences and patterns which were divided into two groups. i.e., virulent and avirulent. Triclosan was found to interact with crucial proteins in *S. mutans* and *E. faecalis*. Triclosan interacts with more than ten important proteins in both the pathogen, as shown by the virulence properties of 8 virulent proteins in *S. mutans* and 5 virulent proteins in *E. faecalis* in the VirulentPred findings. Most of the virulent proteins identified were transcriptional regulators in *S. mutans* and proteins involved in cellular processes in case of *E. faecalis* (Table 1; Fig 1). Several epitopes were identified in the virulent protein, conserved hypothetical protein PksD of *Streptococcus mutans* (Figs 2 and Chart 1).

Target identification is an important component for the development of therapeutic and diagnostic markers for metabolic, infectious [20-25], autoimmune [26,27,28] and systemic disorders. Computational tools have long been used for the purpose of rapid identification of these potential targets from several sources [29-30]. Dental caries being a serious illness in children has to be identified at the right time and treated using drugs that do not give rise to resistant forms [31-34]. Hence, proper identification of the potential targets has become the need of the hour. We investigated the molecular targets of triclosan and their associations with pathogens in this research, which shows that there are interactions with microbes and virulence factors associated with its functions. Triclosan has been integrated with a number of other dental materials to improve inhibitory effects on microbial metabolism in plaque, calculus, and gingivitis accumulation. As triclosan is used at low concentrations, it inhibits microorganism development; however, when used at higher concentrations, it can disrupt the growth of microorganisms. While the *in silico* methods used provide preliminary evidence on the underlying molecular interaction between the compound and protein network of dental pathogens such as *S. mutans* and *E. faecalis*. The analysis has some limitations, such as (a) the bonding between the compound and the pathogen’s protein may be purely physical and (b) the proteins of the red-complex bacteria attacked by the compound may resemble host proteins. To prevent undesirable associations of triclosan with host proteins, in vitro and in vivo studies must be performed to obtain clarification on the healthy use of chemical-compounds on human hosts.

4. CONCLUSION

This study identified molecular targets of triclosan on *S. mutans* and *E. faecalis* which have to be further validated to confirm the critical pathway triggered by the drugs in the physiological conditions. To the best of our knowledge, this study is the first of its kind which aims in understanding the molecular targets of the pathogens against specific drug compounds. The dosage of the drug, minimum inhibitory concentration, and minimum bactericidal concentration against specific microbes should be ascertained by *in vitro* and *in vivo* studies.

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**CONSENT**

Not applicable.

**ETHICAL APPROVAL**

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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683