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Virtual Screening to Identify the Protein Network Interaction of Triclosan in Red Complex Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author NVHR carried out the literature search, data collection, data analysis and manuscript writing. Author JVP has conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. Authors PSG and ASSG equally contributed in the validation and development of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Antimicrobial drug resistance is the major problem encountered world-wide. Novel therapeutic leads have been identified and are regularly tested for their activity against microbial pathogens.
Aim: To identify the protein network interactions of triclosan in red complex pathogens.
Materials and Methods: The present study follows an observational study design which aims to screen for the interaction of triclosan in red complex pathogens. The interaction was analyzed using the STITCH v.5 pipeline. The functional class of proteins identified were assessed using VICMPred and VirulentPred softwares. The microbial pathogens *Treponema denticola ATCC 35405, Tannerella forsythia ATCC 43037, Porphyromonas gingivalis ATCC 33277* are the strains of red complex pathogens that are included in the present study.

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Results and Discussion: Several proteins were found to interact with triclosan. Among the protein interactions, interactions of triclosan with virulent proteins seems to have a greater impact. The NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], Putative NAD dependent epimerase [PGN_1886], GDP-fucose synthetase [PGN_1079], Probable oxidoreductase [PGN_1360] of *Porphyromonas gingivalis*, Conserved hypothetical protein [TDE_2401], Epimerase/dehydratase family protein [TDE_1439] of *Treponema denticola,* NAD dependent epimerase/dehydratase family protein [BFO_2919], Hypothetical protein [BFO_1782], Nitroreductase family protein [BFO_1604] and Nitroreductase family protein [BFO_1516] *Tannerella forsythia* were found to be exhibit virulence nature.
Conclusion: This study identifies the molecular targets of triclosan on red complex pathogens. As triclosan interacts with the red complex pathogens, in future it can be used as a primary medicine for periodontitis and some oral conditions.

Keywords: Triclosan; protein network interaction; red complex pathogens; periodontitis; novel targets.

1. INTRODUCTION

Periodontitis is the most prevalent problem encountered in the dental settings. Periodontitis can be classified as adult periodontitis, rapidly progressive periodontitis and refractory adult periodontitis [1]. The organisms most commonly associated with periodontitis are *Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Pre-votella intermedia, Streptococcus intermedia, Eubacterium nodatum,* etc. Apart from these organisms the red complex pathogens seems to be the major culprits behind periodontitis. Protein network interactions are statistical presentations of contact between proteins in a cell and the drug [2,3]. Protein network interaction will represent both the stable and transient interactions [3].

Triclosan was first reported to be effective against bacteria and fungi. Mouthwash, bar soap, liquid soap, shower gels, face washes/cleansers, hair shampoos, underarm deodorants, shaving creams, after-shave lotion, and anti-acne preparations all now contain triclosan [4]. It has been a widely approved broad spectrum antimicrobial agent which is successful in opposition to many gram negative and gram positive bacteria [5]. The anti gingivitis and antiplaque effectiveness of triclosan in containing debris is accepted. Our team has extensive knowledge and research experience that has translate into high quality publications [6–10].

The emergence of a newer drug resistant community has largely hampered the process of therapy [11,7,8,12]. It is useful as antiseptics to destroy the bacteria on the surfaces of skin and also used in medical devices to prevent the products from microbial decay [9]. It prevents bacterial fatty acid synthesis. Fab pathway is a main target for antimicrobial agents [13]. Several synthetic and phytocompounds have been assessed using in silico methods to identify potential protein targets in common dental pathogens [14,15]. The present study is to identify the protein network interaction of triclosan in red complex pathogens

2. MATERIALS AND METHODS

2.1 Study Design

The present study follows an observational study design which aims to screen for the interaction of triclosan in red complex pathogens. The interaction was analysed using STITCH v.5 pipeline [16]. The functional class of proteins identified were assessed using VICMPred [17] and VirulentPred softwares [18]. The microbial pathogens *Treponema denticola ATCC 35405, Tannerella forsythia ATCC 43037, Porphyromonas gingivalis ATCC 33277* are the strains of red complex pathogens that are included in the present study.

2.2 Prediction of Protein-drug Interactions

To predict the interactions between proteins and chemicals STITCH database (Version 5; 2016) is used. The interactions include associations of direct or physical and indirect or functional is used for the computational prediction and from the responses the data is aggregated. The repertoire of proteins which interacts with *T. forsythia, P. gingivalis* and *T. denticola* and were further used for predicting virulence [16].

2.3 Virulence Prediction

For the identification of virulence factors the software used was VICM pred [17] and Virulent

Pred [18] pipelines. VICMpred groups proteins are classified into four major classes: proteins involved in metabolism, information storage, virulence and cellular processes. The overall accuracy of VirulentPred servers and VICMpred were 86% and 70.75%, respectively.

2.4 Prediction of Subcellular Localization of the Virulent Proteins

The novel drug targets plays an important role in an antimicrobial drug which targets the virulent protein. The subcellular localization of proteins aids in designing using the Computational prediction. The great interest is that cell surface proteins can be used in making vaccines. An algorithm which assigns a probable localization site to a protein from an amino acid sequence is pSORTb V3.0 [19].

2.5 Prediction of B-cell Epitopes in the Virulent Proteins

For the prediction of B-cell epitopes from a protein sequence the server is BepiPred-2.0 was used. To be part of an epitope the residues with scores above the threshold (>0.5) [20,21].

3. RESULTS AND DISCUSSION

Pathogens of the red complex, such as *P. gingivalis, T. denticola, and T. forsythia*, are important contributors to periodontal infections. The removal of these pathogens from the infection site is still a challenge. In silico tools have been largely used to cut down the primary cost of screening active molecules for their antimicrobial effect [22]. Gomez, et al investigated the inhibitory and lethal effect of triclosan against several microorganisms at different stages of their phase of population growth. Several proteins were found to interact with triclosan [20]. Among the protein interactions, interactions of triclosan with virulent proteins seems to have a greater impact (Fig. 1). The NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], putative NAD dependent epimerase [PGN_1886], GDP-fucose synthetase [PGN_1079], probable oxidoreductase [PGN_1360] of *Porphyromonas gingivalis*, conserved hypothetical protein [TDE_2401], Epimerase/dehydratase family protein [TDE_1439] of *Treponema denticola,* NAD dependent epimerase/dehydratase family protein [BFO_2919], hypothetical protein [BFO_1782], nitroreductase family protein [BFO_1604] and nitroreductase family protein [BFO_1516] *Tannerella forsythia* were found to be exhibit virulence nature (Table 1). Most of the proteins identified as virulent were located in the cytoplasm (Table 2). Several virulent proteins identified were found to possess multiple epitopes demonstrating a greater tendency to elicit immune response in the host (Fig. 2).

Several *in silico* studies have been performed to predict the potential targets of microbial pathogens against the drug selected [21-23]. Farsi and Tanner, performed an in vitro study to analyse the resistance of *Porphyromonas gingivalis, Prevotella intermedia, and Tannerella forsythia* to triclosan. No growth of *Porphyromonas gingivalis* and *P. intermedia* were observed in plates containing ≥ 2 µg/ml triclosan, while *T. forsythia* did not grow on ≥ 1.66 µg/ml. Resistant strains of *P. intermedia* triclosan developed after prolonged incubation at 2 µg/ml [24]. Our research team has performed several studies related to computational analysis related to infectious diseases, metabolic, autoimmune disorders and cancer [25-26].

Table 1. Proteins of red complex pathogens interacting with triclosan

Organism	Identifier	Proteins which interacts with triclosan	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
Porphyromonas gingivalis	PGN_1370	NAD-dependent nucleotide-diphosphate-sugar epimerase	Cellular process	Virulent	0.6274
	PGN_0224	UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase	Cellular process	Avirulent	-1.372
	PGN_1886	NAD dependent epimerase	Cellular process	Virulent	0.9781
	PGN_1079	GDP-fucose synthetase	Metabolism Molecule	Virulent	1.0619
	PGN_0365	Arginyl-tRNA synthetase	Cellular process	Avirulent	-1.099
	PGN_1652	Nitroreductase	Cellular	Avirulent	-1.006

Organism	Identifier	Proteins which interacts with triclosan	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
			process		
	PGN_0765	Nitroreductase	Metabolism Molecule	Avirulent	-1.053
	PGN_1360	Oxidoreductase	Metabolism Molecule	Virulent	1.0723
Treponema denticola	TDE_2401	Hypothetical protein	Metabolism Molecule	Virulent	0.7963
	TDE_0708	Nitroreductase	Metabolism Molecule	Avirulent	-0.405
	TDE_1439	Epimerase/dehydratase	Cellular process	Virulent	0.9466
	TDE_1363	Nitroreductase	Metabolism Molecule	Avirulent	-1.074
	TDE_1953	TetR family transcriptional regulator	Metabolism Molecule	Avirulent	-1.081
	TDE_0246	TetR family transcriptional regulator	Cellular process	Avirulent	-0.345
Tannerella forsythia	BFO_2919	NAD dependent epimerase/dehydratase family protein	Cellular process	Virulent	0.7052
	BFO_1051	Nucleotide sugar dehydrogenase	Cellular process	Avirulent	-0.113
	BFO_3081	GDP-L-fucose synthetase	Metabolism Molecule	Avirulent	-0.234
	BFO_3174	Nitroreductase family protein	Cellular process	Avirulent	-1.062
	BFO_1782	Hypothetical protein	Cellular process	Virulent	0.7487
	BFO_1604	Nitroreductase family protein	Metabolism Molecule	Virulent	1.0084
	BFO_1516	Nitroreductase family protein	Cellular process	Virulent	1.0676
	BFO_1640	Arginine--tRNA ligase	Metabolism Molecule	Avirulent	-1.055
	BFO_0712	Nitroreductase family protein	Metabolism Molecule	Avirulent	-0.984
	BFO_1683	Short chain dehydrogenase/reductase family oxidoreductase	Metabolism Molecule	Avirulent	-0.924

Table 2. Subcellular location of virulent proteins from red complex pathogens

Virulent protein	Subcellular location	Score
NAD-dependent nucleotide-diphosphate-sugar epimerase	Cytoplasmic	8.96
Putative NAD dependent epimerase	Cytoplasmic	8.96
GDP-fucose synthetase	Cytoplasmic	9.97
Probable oxidoreductase	Unknown	-
Epimerase/dehydratase	Cytoplasmic	8.96
Conserved hypothetical protein	Cytoplasmic	8.96
NAD dependent epimerase/dehydratase family protein	Cytoplasmic	8.96
Hypothetical protein BFO_1782	CytoplasmicMembrane	9.82
Nitroreductase family protein	Unknown	-
Nitroreductase family protein	Cytoplasmic	8.96

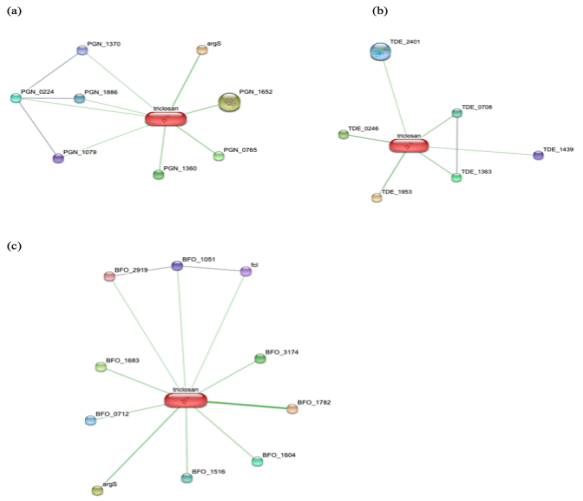

Fig. 1. Protein interaction network of (a) *Porphyromonas gingivalis (b) Treponema denticol a and (c) Tannerella forsythia* with triclosan

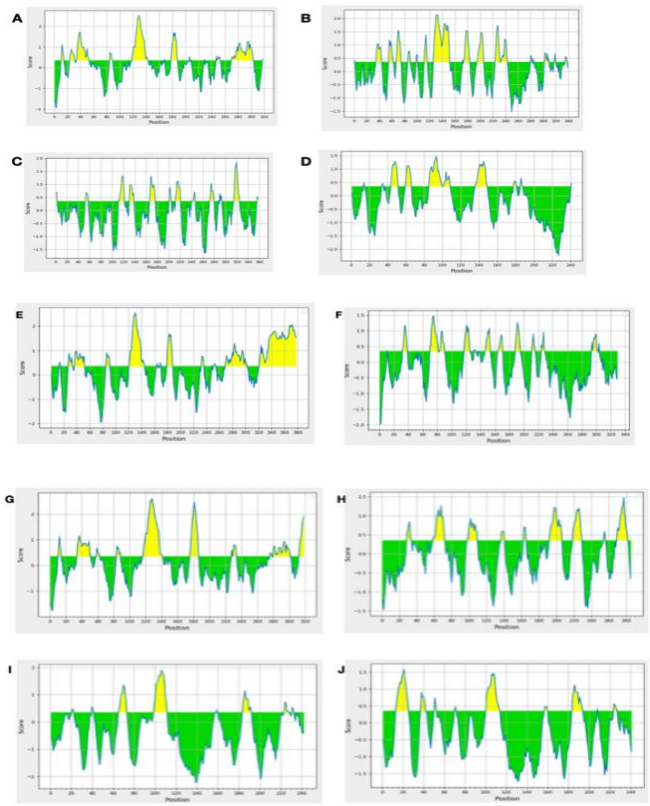

Fig. 2. Predicted epitopes in virulent proteins (A) NAD-dependent nucleotide-diphosphate-sugar epimerase [PGN_1370], (B) Putative NAD dependent epimerase [PGN_1886], (C) GDP-fucose synthetase [PGN_1079], (D) Probable oxidoreductase [PGN_1360] of *Porphyromonas gingivalis*, (E) Conserved hypothetical protein [TDE_2401], (F) Epimerase/dehydratase family protein [TDE_1439], *Treponema denticola* (G) NAD dependent epimerase/dehydratase family protein [BFO_2919], (H) Hypothetical protein [BFO_1782], (I) Nitroreductase family protein [BFO_1604], (J) Nitroreductase family protein [BFO_1516] *Tannerella forsythia*

4. LIMITATIONS

The limitations of the present study is that the protein interactions demonstrated here may not work the same way in a complex biological system. Also, sometimes the microbial proteins mimicking host proteins could bring about cross reactions with the bioactive compound.

5. FUTURE SCOPE

Computational methods have supported basic science researchers by cutting down the time required for analysis of numerous bioactive compounds and screening the best suitable compound which works against specific pathogens. The study can be further extended in an *in vitro* and *in vivo* set up to provide more evidence on the antimicrobial effect of the compound against dental pathogens.

6. CONCLUSION

This study identifies the molecular targets of triclosan on red complex pathogens. As triclosan interacts with the red complex pathogens, in future it can be used as a primary medicine for periodontitis and some oral conditions.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is 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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