SCREENING AND IN SILICO VALIDATION OF ANTIMICROBIAL PEPTIDES DERIVED FROM DNASE1 AND DNASE 1- LIKE 3

S.Kesava Priya [1], J.Vijayashree Priyadharsini [2*], A.S. Smiline Girija [3], P. Sankar Ganesh [4]
[1] Saveetha dental college and Hospital, Saveetha Institute of Medical and technical sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
[2] Associate Professor, Department of Microbiology, Saveetha dental college and Hospital, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India. vijayashreej.sdc@saveetha.com
[3] Professor, Department of Microbiology, Saveetha dental college and Hospital, Saveetha Institute of Medical and technical sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
[4] Assistant Professor, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India. sankarganeshp.sdc@saveetha.com

Abstract
Introduction: Drug resistance is emerging as a potential threat to mankind. Numerous compounds from diverse sources have been tested against potential drug resistant pathogens. Antimicrobial peptides are small molecules used in peptide-therapy. The present study is one such attempt to identify peptides from deoxyribonucleases and demonstrate its antimicrobial activities employing *in silico* tools.
Aim: To demonstrate putative antimicrobial activity of *DNASE1* and *DNASE1L3* derived antimicrobial peptides.
Materials and methods: The observational study employs the use of computational tools which was used to identify peptide molecules and demonstrate their antimicrobial properties such as anti-biofilm, anti-fungal etc., Furthermore, the toxicity and cell penetrating properties of peptides were evaluated.
Results and discussion: Among three peptides of DNase1-like 3 two were found to exhibit active anti-biofilm and anti-fungal property. Whilst, DNAase 2 was found to show only antifungal property. None of the proteins identified were found to be cell penetrating or toxic in nature.
Conclusion: Taken together, the antimicrobial peptides derived from DNase1-like 3 and DNAase 2 were found to exhibit promising antimicrobial effects as predicted by the *in silico* tools. Further validation of the peptides using *in vitro* and *in vivo* models would aid us in understanding the mechanism of action of these small molecules in a complex biological environment.
Keyword: Deoxyribonuclease, antimicrobial peptides, *in silico,* anti-biofilm, anti-fungal, novel targets.

INTRODUCTION

The emergence and resurgence of drug resistant pathogens continues to threaten our ability to treat common infections. The rapid spread of multi- and pan drug resistant organisms poses a significant threat to mankind globally, by hampering the treatment of infections with currently available antibiotics(Moström *et al.*, 2021). Several drug resistant species have been documented in dental settings also. These microbes in association with other microorganisms of the oral cavity tend to worsen the conditions of dental diseases (Ramalingam, Selvi and Jayaseelan, 2019; Jaisankar *et al.*, 2020; Jayaseelan and Arumugam, 2020; Jayaseelan and Paramasivam, 2020; Kumar *et al.*, 2020; Mathivadani, Smiline and Priyadharsini, 2020; Teja and Ramesh, 2020; Barma *et al.*, 2021; Girija and Smiline Girija, 2021; Kadanakuppe and Hiremath, 2021; Samuel, 2021; Samuel *et al.*, 2021; Ushanthika *et al.*, 2021)and make them refractory to treatments (Priyadharsini *et al.*, 2018b; Vijayashree Priyadharsini, 2019). Indiscriminate use of antibiotics is found to be the major reason behind such a rising trend in drug resistance.

The present situation underscores the need for identifying novel bioactive compounds or antimicrobial peptides for therapeutic use. Synthetic (Paramasivam, Vijayashree Priyadharsini and Raghunandhakumar, 2020), semi-synthetic and phytocompounds (Priyadharsini *et al.*, 2018a; Paramasivam and Vijayashree Priyadharsini, 2020; Paramasivam, Priyadharsini and Raghunandhakumar, 2020) have been tested for decades to establish their antimicrobial activity against dental pathogens. (Krishnan and Lakshmi, 2013; Soh and Narayanan, 2013; Lekha *et al.*, 2014; Dhinesh *et al.*, 2016; PradeepKumar *et al.*, 2016)

The present study is one such attempt to identify peptides and demonstrate the role of these peptides derived from.

METHODOLOGY

The antimicrobial proteins DNAase 2 and DNase1-like 3 were selected for the study based on an extensive text mining process. The protein sequences of the proteins were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (https://www.ncbi.nlm.nih.gov/protein/).

Antimicrobial peptide identification

Antimicrobial peptide analysis (AMPA) is a web based application employed for identifying and assessing the

antimicrobial domains in a protein. The source is used to design and develop peptide based drugs against microbial pathogens (Torrent *et al.*, 2012) (Torrent, Nogués and Boix, 2009).

Anti-biofilm property
dPABB (design Peptides Against Bacterial Biofilms) algorithm is based on the SVM and Weka models used to identify anti-biofilm peptides based on their amino acid composition, selected residue and position of the residues. The scores generated for each of the peptide molecules are then used to ascertain the anti-biofilm property (Sharma *et al.*, 2016).

Antifungal property
The tool used in silico prediction of antimicrobial peptides for its antifungal property is Antifp. The module allows users to predict single or multiple sequences for its antifungal properties. The tool can be used for designing peptides and scanning protein sequences to identify peptides and their mutant analogs followed by the screening for antifungal property (Agrawal *et al.*, 2018).

Cell penetrating property
Identification of newer peptide molecules with the ability to penetrate cells using high throughput methods is known to consume time as well as labour. The in silico screening procedures coupled with experimental validation is considered to be more feasible and cost-effective. The results could be replicated in in vitro conditions with much ease and confidence. CellPPD is one such standalone application developed to predict and design cell penetrating peptide molecules (Gautam *et al.*, 2013) (Gautam *et al.*, 2015).

Toxicity prediction
Prediction of toxicity of peptides is a vital step in designing antimicrobial peptides. The ToxinPred tool has been used in the present study. The algorithm identifies certain amino acid residues such as Cys, His, Asn and Pro and their placements at various positions which makes them toxic. ToxinPred can be used to predict whether the designed peptide is toxic or non-toxic, consequences of mutations on toxicity and identification of toxic regions in a protein (Gupta *et al.*, 2013).

RESULTS AND DISCUSSION
Antimicrobial drug resistance and biofilm formation are the two most important challenges faced by the clinicians. The treatment of the most simplest infections could be hampered by the rise in resistant species. In line with the above facts, two proteins, DNAase 2 and DNase1-like 3 were selected for the present study. Among three peptides of DNase1-like 3 two were found to exhibit active anti-biofilm and anti-fungal properties. Whilst, DNAase 2 was found to show only antifungal property (Table 1). The physicochemical properties of the peptides identified are given in Table 2. None of the proteins identified were found to be cell penetrating or toxic in nature. Tetz *et al.,* demonstrated that DNAses could effectively inhibit biofilms formed by various gram positive and gram negative bacteria. The extracellular DNA excluded during cell destruction is cleaved by DNAses, which alters the biofilm in terms of biomass, morphology, architecture and number of colony forming units. The altered biofilm improves penetration of antibiotics. The team observed that addition of DNases enhanced the effectiveness of antibiotics resulting in the decrease of biomass and CFU.

Another study conducted by Tets *et al.(Waryah et al., 2017),* identified the effect of antibiotics in combination with DNAse on biofilms of unrelated bacterial pathogens isolated from patients with inflammatory urinary diseases. They showed that destruction of extracellular DNA by DNAses made biofilms more vulnerable to antibacterial drugs. A very recent study by Liu *et al.(Baelo et al., 2015),* used polymer encapsulated DNAse against biofilm. Crystal violet staining results demonstrated disintegration of biofilm (92.2%). Thus the study opened new avenues towards the development of a strategy for treating biofilm associated infections. Accumulating evidence has proved the activity of DNAses as potent antimicrobial agents. The study is first of its kind wherein peptide derivatives of DNAses have been analyzed to reveal the anti-microbial effect.

LIMITATIONS
The limitations of the present study design are (a) the functions of the peptides described here are predictive of the antimicrobial protein studies using in silico tools which may or may not exhibit the same effect in a complex biological environment (b) the protein interactions and pathway involved in causing deleterious effect to the microbes should be investigated using experimental procedures

FUTURE SCOPE
The study presented here can be replicated using appropriate experimental designs so as to prove their effectiveness against dental pathogens. Administration of antimicrobial peptides would be a best alternative to the synthetic and semi-synthetic drugs available for current usage for which most of the pathogens have gained resistance.

CONCLUSION
Biofilm formation has been associated with the difficulty in treating the infection associated with it. Furthermore, biofilm forming organisms have also been found to exhibit drug resistance. Hence, the antimicrobial peptides identified in the present study have to be evaluated using *in vitro* and *in vivo* models to ascertain their antimicrobial activity against most of the dental pathogens.

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Conflict of interest
None

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Table 1: The list of antimicrobial peptides predicted from DNAase 2 and DNase1-like 3 their anti-biofilm and anti-fungal properties

Antimicrobial protein	Antimicrobial peptide	Anti-biofilm property	SVM score	Anti-fungal property	SVM Score	Cell penetrating property	Toxicity
DNase1-like 3	NSRRGITYNYVISSRLGRNTYK	Inactive	-0.61	Non-antifungal	-0.249	Non-CPP	Non-toxin
	VPKKAWKNIRLRT	Active	1.66	Antifungal	0.437	Non-CPP	Non-toxin
	RAFTNSKKSVTLRKKTKSKR	Active	0.16	Antifungal	0.472	Non-CPP	Non-toxin
DNAse 2	MGKQLTYTYPWVYNY	Inactive	-0.37	Antifungal	0.109	Non-CPP	Non-toxin

Table 2: Physiochemical properties of the antimicrobial peptides

Antimicrobial peptide	Hydrophobicity	Hydropathicity	Hydrophilicity	Molecular weight
NSRRGITYNYVISSRLGRNTYK	-0.38	-1.09	0.09	2619.26
VPKKAWKNIRLRT	-0.41	-1.01	0.45	1610.17
RAFTNSKKSVTLRKKTKSKR	-0.60	-1.61	1.03	2365.10
MGKQLTYTYPWVYNY	-0.06	-0.70	-0.97	1927.43

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