Original Article

DOI: 10.7860/JCDR/2025/72668.20545

Characterisation of *EfbA* from the Endodontic Pathogen *Enterococcus faecalis* and Prediction of Immunodominant *EfbA* Epitope Peptides: An In-vitro and In-silico Study

Microbiology Section

DEV ARORA[1], AS SMILINE GIRIJA[2], J VIJAYASHREE PRIYADHARSINI[3]

ABSTRACT

Introduction: *Enterococcus faecalis (E. faecalis)* belongs to Group D Streptococci and causes recalcitrant infections such as urinary tract infections, wound infections, intra-abdominal and pelvic infections, bacteraemia, and endocarditis. The emergence of multiple drug resistance, including to the last-resort drug vancomycin, is a major concern with *E. faecalis* infections. Therefore, it is necessary to implement alternative strategies to combat *E. faecalis* infections in dental settings. An immuno-informatics approach is one such strategy that can predict and assess the immunodominant B-cell and T-cell epitopes. Thus, there is a need to identify novel vaccine candidates as immunodominant epitope peptides from the Enterococcal Fibronectin Binding Protein-A (EfbA) protein for *E. faecalis*.

Aim: To predict the immunodominant B-cell and T-cell epitopes from the *EfbA* protein of *E. faecalis*.

Materials and Methods: An in-vitro and in-silico pilot study was conducted in the Department of Microbiology at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. Total of 20 carious scrapings were collected from patients with root caries and were phenotypically characterised for *E. faecalis* and the *EfbA* genetic determinant by Polymerase Chain Reaction (PCR) amplification. The *EfbA* protein was retrieved from the National Center for Biotechnology Information (NCBI) database, after which prediction of antigenicity and allergenicity was performed via the VaXiJen and Algpred servers, respectively. The prediction of T-cell epitopes was carried out with the help of the EpiDOCK server, and B-cell predictions were made using the Kolaskar and Tongaonkar tool. Docking of the epitopes with reference to Human Leukocyte Antigens (HLA) alleles was assessed using the ClusPro server, and the results were evaluated for the T-cell dominant epitopes.

Results: Among the 20 samples, *E. faecalis* was characterised in 5 (25%) patients, with three strains exhibiting Multidrug Resistant (MDR) traits. Among the three MDR strains, two strains showed the presence of the *EfbA* gene by PCR. In-silico analysis of the *EfbA* protein yielded a total of 17 epitopes, and based on the assessments, a final selection of two epitopes (IRAKGKNHK and LLLSAHPSY) was made, showing promising docking scores with HLA alleles and TLR2.

Conclusion: Among the two epitopes, LLLSAHPSY was identified as the most significant immune-dominant epitope predicted, which needs to be further evaluated for vaccine synthesis and experimentation.

Keywords: Oral health, Root caries, Virulence

INTRODUCTION

Enterococci, classified as Group D Streptococci, are transient flora among individuals in healthcare settings and a permanent flora of the gut and oral microbiome. They are associated with various infections, such as bacterial endocarditis, bacteraemia, meningitis and various neonatal infections, some of which may be potentially fatal [1]. *Enterococcus* species are intrinsically resistant to cephalosporins, while their resistance to other drugs is acquired [2]. *E. faecalis* is also associated with several oro-dental diseases, including caries, endodontic infections, periodontitis and peri-implantitis, which can further lead to endocarditis [3]. They are primarily found in secondary endodontic infections, forming biofilms [4] and being a part of the oral microbiome [5].

In recent decades, the emergence of resistant strains has become a major concern in treating *E. faecalis* infections. The transfer of antibiotic resistance genes via horizontal gene transfer between different strains of *E. faecalis*, as well as the presence of vancomycin-resistant strains, has also been reported [6]. Therefore, it is a herculean task to treat infections associated with these drug-resistant strains, highlighting the urgent need to implement strategies alternative to conventional drugs to combat this particular organism.

Reverse vaccinology is an immune-informatics approach that involves analysing multiple genome sequences to identify an antigen or a promising epitope for vaccine synthesis. It is a cost-effective measure that employs various bioinformatics databases and tools to design and evaluate immune-dominant peptides [7]. Several virulence factors associated with *E. faecalis* have been documented, including EfbA, enterococcal surface protein (esp), hyaluronidase (hya) and *E. faecalis* endocarditis antigen A (efaA), which are presumed to be involved in adhesion to host cells, leading to endocarditis [8]. In this study, the EfbA protein was selected, as it is a PavA-like fibronectin adhesion protein that shows a significant association with various recalcitrant infections [9].

The present study adopts an approach targeting a vital virulent protein selected from *E. faecalis*. The *EfbA* protein binds with high affinity to immobilised fibronectin, collagen I and collagen V. Fibronectin is an important component of the extracellular matrix and is exposed when tissue is injured; it also contributes to the thrombogenicity of surfaces.

This study was designed to predict immunodominant B-cell and T-cell epitopes from the *EfbA* protein and to identify the most suitable epitope sequence that can be used for vaccine production, as it plays a crucial role in adhesion to host tissues. Identifying epitopes that can serve as immunogens is the first stage in the vaccine development process. The predicted epitopes, which exhibited high antigenicity, non-allergenicity, non-toxicity and favourable

immunogenicity scores, were evaluated for further immunogenicity analysis in-vivo. The physicochemical, structural and immunological properties of the resultant vaccine construct were examined using various bioinformatics tools. The aim of this study was, therefore, to assess the frequency of *EfbA* genetic determinants among clinical strains of *E. faecalis* and to predict effective in-silico based immune-dominant epitopes against *EfbA* of *E. faecalis*.

MATERIALS AND METHODS

This in-vitro and in-silico pilot study was conducted in the Department of Microbiology at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, to characterise drug-resistant *E. faecalis* and to predict immune-dominant epitopes through in-silico analysis. The study was conducted from April 2022 to June 2022 over a three-month period. Informed consent, along with institutional review and ethical clearance, was obtained before initiating the study (SRB/SDC/UG-2004/21/MICRO/058; IHEC/SDC/UG-2004/21/MICRO/599).

Inclusion criteria: Patients with typical root caries (n=20), as determined by an endodontist, were included in the study.

Exclusion criteria: Patients with surface or smooth caries and those who had received any previous antibiotic medications were excluded from the study.

Excavated carious scrapings from the root caries were collected as samples from the patients. The carious dentine was immediately transferred to the microbiology laboratory after being collected in sterile trypticase soy broth. The materials were plated onto sterile Brain Heart Infusion (BHI) agar and incubated for 24 hours at 37°C. After incubation, the colonies were identified using colony morphology and Gram staining was performed to determine the morphology of the bacterium. Phenotypic characterisation was done using catalase and bile esculin tests. The strains were also subjected to antibiogram profiling using Clinical and Laboratory Standards Institute (CLSI) guidelines [9], with the recommended antibiotics. The antibiotics tested included penicillin (10 units), ampicillin (10 µg), vancomycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), doxycycline (30 µg), minocycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and rifampicin (5 µg), using the agar disc diffusion method to profile the isolates for susceptibility and resistance patterns.

Genotypic characterisation of *EfbA* gene in *E. faecalis*: Fresh cultures of *E. faecalis* that were MDR were retrieved after 24 hours of incubation at 37°C on fresh BHI agar plates. Following the manufacturer's instructions (Qiagen kit), genomic DNA was extracted from the *E. faecalis* strains. Amplification by PCR was performed by mixing 7.8 µL of 2x master mix (Taraka, Japan) with 5.6 µL of Distilled Deionised Water (DDW) to detect the presence of *EfbA*. A 15 µL reaction mixture was prepared using specific primers for *EfbA* (F: GCACAAGTCCCAAAAGGAGC and R: AAGTGCGGCTTCAGTAAGGG) (0.31 µL of 100 pmol/mL concentration; Eurofins Genomic India Pvt., Ltd., Bangalore). Germany's Eppendorf thermocycler was used to accomplish 35 cycles of PCR amplification at an annealing temperature of 58°C. The amplicon was then observed on a 1% agarose gel electrophoresis with Ethidium Bromide (EtBr) and visualised using a gel documentation system. The size was determined using a 1.5 Kbp DNA ladder.

Retrieval of *EfbA* and its secondary structure prediction: The *EfbA* protein sequence was retrieved from the NCBI database and the subcellular location was predicted using the CELLO v.2.5 subcellular localisation prediction tool. The secondary structure of the *EfbA* protein was predicted using the Self-Optimised Prediction Method with Alignment (SOPMA) server.

Evaluation of antigenicity and allergenicity: The antigenicity of the *EfbA* protein was predicted using the VaxiJen v2.0 server. The physicochemical characteristics of the predicted epitopes were determined using alignment-based approaches. The web server AlgPred, which helps predict epitopes that may cause allergies, was used to evaluate the allergenicity of the *EfbA* protein. The IgE mapping for the predicted epitopes was set using the server's default settings.

Prediction of T-cell Major Histocompatibility Complex (MHC) class II binders: T-cell-MHC class II molecule interactions are vital in initiating antigen-specific immune responses. The *EfbA* epitopes associated with T-cell interactions were assessed using the EpiDOCK server, which predicts the binding of epitopes to HLA-DR (12 alleles), HLA-DQ (6 alleles) and HLA-DP (5 alleles). The input sequence was provided in FASTA format to identify the maximum number of epitope binders (≥10) for further analysis.

Antigenicity and stability prediction of selected T-cell epitopes: The shortlisted epitopes were assessed for their antigenicity using the VaxiJen v2.0 server and their stability index was evaluated using the ProtParam server with the default parameters.

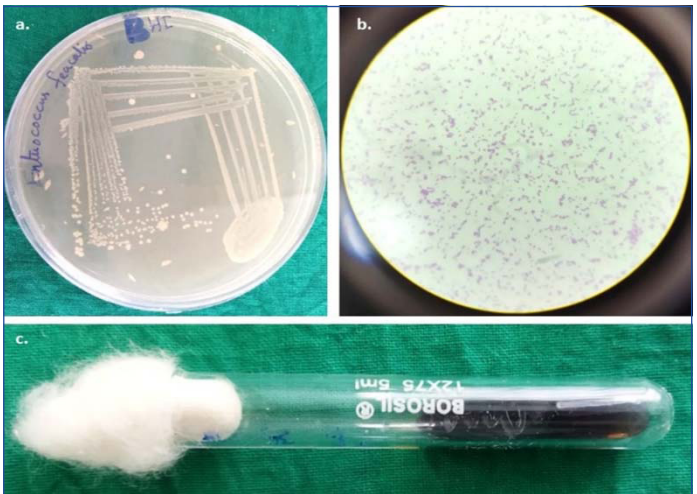
Validation of the predicted structures of the epitopes: The structures of the predicted MHC Class II binders were predicted using the PepFold server. The predicted structures were validated using the RAMPAGE tool, which predicts the stereochemical properties of the given structure.

Molecular docking of epitopes with HLA alleles using ClusPro server: The three-dimensional structures of HLA-DP (3LQZ), HLA-DQ (5 KSV), HLA-DR (4AH2) and TLR-2 (6NIG) were retrieved from the PDB database. The molecular docking analysis of the predicted MHC binders with the HLA alleles was carried out using the ClusPro server.

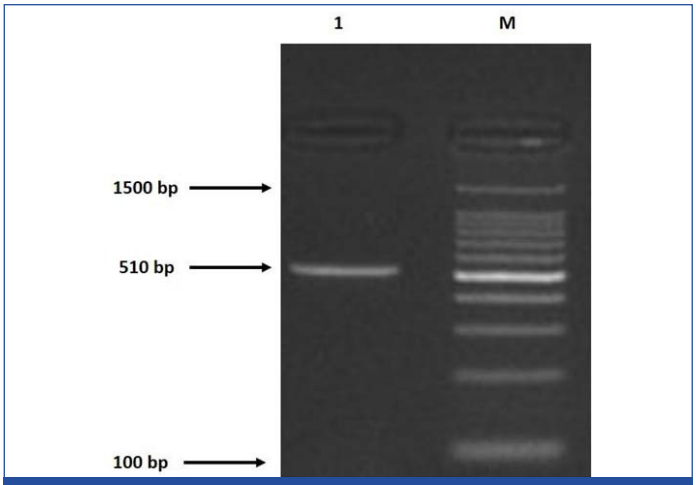
B-cell epitope prediction: The B-cell epitope prediction of the *EfbA* protein was carried out using the Immune Epitope Database (IEDB) with the Kolaskar & Tongaonkar Antigenicity Prediction method.

RESULTS

Characterisation of *E. faecalis*: Out of the total of 20 samples examined, five strains were characterised as *E. faecalis*. The *E. faecalis* colonies were identified by their minute pinpoint colonies on the BHI agar plates. *E. faecalis* was identified by the typical diplococci, which are gram-positive oval cocci, exhibiting a negative catalase test and a positive bile esculin test [Table/Fig-1]. Colonies were identified as MDR strains based on the antibiogram susceptibility profile, showing a resistance pattern for more than three different tested antibiotic groups. Two of the three MDR strains tested positive for the *EfbA* gene, with amplicons that were 510 bp in size [Table/Fig-2].



[Table/Fig-1]: Phenotypic characterisation of *E. faecalis* from the samples: a) BHI agar plate showing the growth of *E. faecalis*; b) Gram stain showing the gram positive diplococci in oval shape; c) Bile esculin agar showing positive for the isolated strain of *E. faecalis*.



[Table/Fig-2]: Electrophoregram of *EfbA* gene product of size 510 bp in lane 1 with 1.5Kbp marker lane (M).

Prediction of epitope peptides against *EfbA* protein of *Enterococcus faecalis*: The *EfbA* protein selected for the study was subjected to various in-silico analysis to predict its parameters and determine the dominant T-cell and B-cell epitopes. The parameters included predictions of the secondary structures, antigenicity, stability, location, allergenicity, validation of the structures and final drug-ligand interactions.

Secondary structure, antigenicity and stability predictions of the T-cell epitopes: The projected epitopes contained 32.98% random coils, 6.67% beta turns, 17.19% extended strands and 43.16% alpha helices. The antigenicity and stability of the peptide binders (≥10) were analysed. Five peptides were selected from the 17 shortlisted peptides with values greater than 0.4 and stability scores above 40, according to the established parameters of the ProtParam server [Table/Fig-3].

Position of peptide	Sequence	Number of binders to HLA alleles (DP, DQ, DR)	Vaxijen v 2.0	Protparam
1	MSFDGVFTH	11	0.1845	71.42
3	FDGVFTHSM	13	0.2214	22.60
7	FTHSMVHEL	15	0.0288	8.89
11	MVHELTETL	13	-0.3100	30.29
20	VSGRISKIH	10	1.2837	13.17
23	RISKIHQPY	10	-0.3538	34.57
24	ISKIHQPYE	11	-0.8518	26.19
31	YENEVVLVI	16	-0.3772	0.51
36	VLVIRAKGK	14	-0.3054	-9.98
38	VIRAKGKNH	12	0.3423	-9.98
39	IRAKGKNHK	16	1.4842	16.33
42	KGKNHKLLL	14	1.1740	6.90
48	LLLSAHPSY	11	0.6974	17.66
50	LSAHPSYAR	10	0.7999	43.97
53	HPSYARIQL	11	0.5605	53.40
56	YARIQLSTI	13	0.3553	35.20
58	RIQLSTITY	13	0.7903	8.89

[Table/Fig-3]: Antigenicity prediction of selected T-cell epitopes based on antigenicity and stability analysis prediction.

Location, antigenicity and allergenicity of the *EfbA* protein: The NCBI database was searched for the *E. faecalis EfbA* protein and the sequence ID was WP 192203412.1. The CELLO v.2.5 subcellular localisation prediction tool indicated that the subcellular location would be either cytoplasmic (2.230) or membrane (2.198). The predicted antigenicity of *EfbA* was found to be 0.3592 using the VaxiJen v2.0 server and the protein's projected non-allergenicity was assessed using the AlgPred server [Table/Fig-4,5].

Position of peptide	Sequence	Number of binders to HLA alleles	HLA alleles	
			DP, DQ, DR	Threshold value
20	VSGRISKIH	10	DPA1*0201/DPB1*0501	0.192
			DQA1*0101/DQB1*0501	0.217
			DQA1*0102/DQB1*0602	0.343
			DQA1*0401/DQB1*0402	0.519
			DQA1*0501/DQB1*0201	0.387
			DQA1*0501/DQB1*0301	0.449
			DRB1*0404	0.569
			DRB1*0405	0.429
			DRB1*0802	0.305
			DRB1*1302	0.576
39	IRAKGKNHK	16	DPA1*0103/DPB1*0201	0.172
			DPA1*0103/DPB1*0402	0.274
			DPA1*0201/DPB1*0501	0.437
			DQA1*0101/DQB1*0501	0.392
			DQA1*0102/DQB1*0602	0.685
			DQA1*0301/DQB1*0302	0.297
			DQA1*0401/DQB1*0402	0.523
			DQA1*0501/DQB1*0201	0.401
			DRB1*0101	0.469
			DRB1*0401	0.567
			DRB1*0404	0.738
			DRB1*0405	0.732
			DRB1*0701	0.3
			DRB1*0802	0.802
			DRB1*1302	0.94
42	KGKNHKLLL	14	DPA1*0103/DPB1*0201	0.131
			DPA1*0201/DPB1*0101	0.07
			DPA1*0201/DPB1*0501	0.652
			DQA1*0101/DQB1*0501	0.237
			DQA1*0102/DQB1*0602	0.44
			DQA1*0501/DQB1*0201	0.234
			DRB1*0101	0.352
			DRB1*0301	0.762
			DRB1*0404	0.72
			DRB1*0405	0.341
			DRB1*0802	0.663
			DRB1*0901	0.4
			DRB1*1302	0.41
			DRB1*1501	0.467
48	LLLSAHPSY	11	DPA1*0103/DPB1*0201	0.241
			DQA1*0401/DQB1*0402	0.253
			DRB1*0301	0.857
			DRB1*0404	0.933
			DRB1*0405	0.292
			DRB1*0701	1.08
			DRB1*0802	0.475
			DRB1*0901	0.927
			DRB1*1101	0.784
			DRB1*1201	1.115
			DRB1*1302	0.539
58	RIQLSTITY	13	DPA1*0201/DPB1*0501	0.415
			DQA1*0301/DQB1*0302	0.116
			DQA1*0401/DQB1*0402	0.747
			DQA1*0501/DQB1*0201	0.74
			DQA1*0501/DQB1*0301	0.384
			DRB1*0101	0.32
			DRB1*0404	0.398
			DRB1*0405	0.314
			DRB1*0802	0.558
			DRB1*0901	0.873
			DRB1*1101	0.528
			DRB1*1201	0.79
			DRB1*1302	0.579

[Table/Fig-4]: Prediction of T-cell epitopes of *EfbA* using EPIDOCK server for HLA binders.

	Peptides	Most favoured region
Epitope 1	VSGRISKIH	83.333
Epitope 2	IRAKGKNHK	100
Epitope 3	KGKNHKLLL	83.333
Epitope 4	LLLSAHPSY	100
Epitope 5	RIQLSTITY	85.714

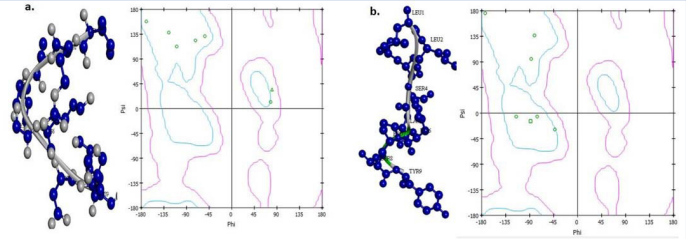
[Table/Fig-5]: Structure prediction of the T-cell *EfbA* epitopes using Pepfold and Ramachandran plot evaluation.

PepFold and Ramachandran plot evaluation for the predicted structures: The structures for all 17 selected epitopes were obtained and the most favoured regions were identified through

the evaluation of the peptides using the Ramachandran plot [Table/Fig-6]. Two epitopes, IRAKGKNHK and LLLSAHPSY, showed 100% favoured regions [Table/Fig-7].

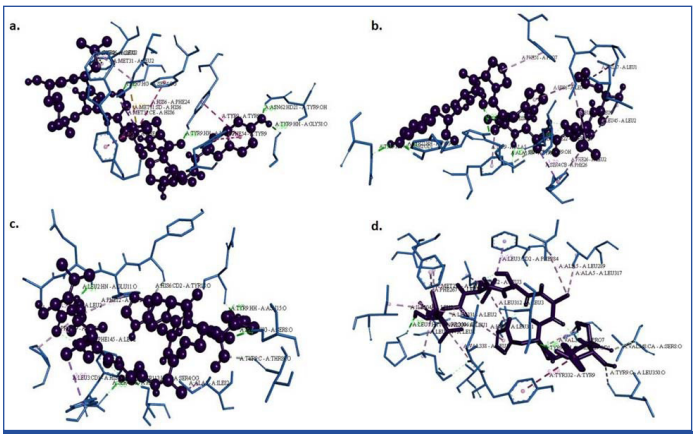
Molecular Docking of epitopes with HLA-alleles	Epitopes	HLA-DP	HLA-DQ	HLA-DR	TLR-2
Epitope 1	IRAKGKNHK	-688.0	-599.8	-635.2	-717.6
Epitope 2	LLLSAHPSY	-851.5	-815.9	-899.0	-1090.3

[Table/Fig-6]: Docking interaction scores of the predicted epitopes with HLA-DP, HLA-DQ, HLA-DR, TLR-2.

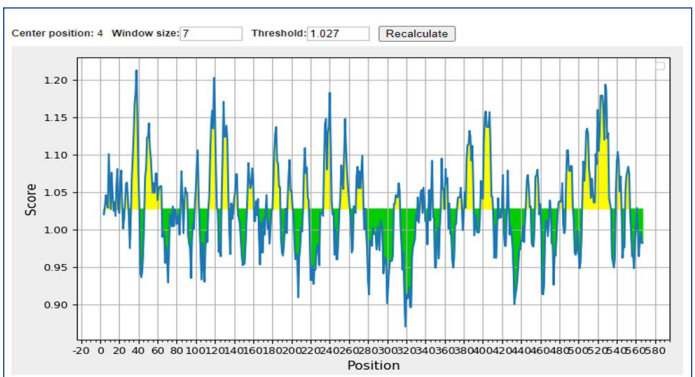


[Table/Fig-7]: Structure predictions of the predicted epitopes using Pepfold server and its validation by Ramachandran plot: a) IRAKGKNHK; b) LLLSAHPSY.

Docking interactions: Molecular docking with HLA-DP, HLA-DQ, HLA-DR and TLR2 is shown in [Table/Fig-8]. The docking interactions are illustrated in [Table/Fig-6-9] for HLA-DP, HLA-DQ, HLA-DR and TLR2, respectively. The B-cell epitope prediction is displayed in [Table/Fig-9]. The X-axis and Y-axis denote the sequence position and antigenic propensity, respectively; the regions above the threshold value are considered antigenic and are depicted in yellow. The yellow peaks represent the antigenic nature of the predicted epitope peptides.



[Table/Fig-8]: Molecular docking of the final predicted epitope LLLSAHPSY with: a) HLA-DP; b) HLA-DQ; c) HLA-DR; and d) TLR2.



[Table/Fig-9]: Kolaskar & Tongaonkar antigenicity predictions of the B cell dominant LILFLIGNY epitope.

DISCUSSION

Enterococci, primarily *E. faecalis*, are the third most common pathogens isolated from native and prosthetic valve endocarditis worldwide. The bacterium has been isolated with a frequency of 12% from surgical wound samples and up to 2% from blood cultures amidst various functional biomes of the oral cavity [10,11]. It is also a potent pathogen associated with urinary tract infections, with diabetes being one of the risk factors [10] and is very common among the recently reported oro-dental pathogens [12]. A previous study characterised the role of *EfbA* in in-vitro adherence to extracellular matrix proteins and its promotion of urinary tract infections. The present study documented the prevalence of *E. faecalis* (25%) in root caries, with 60% of the strains being MDR.

The emergence of MDR enterococci causing endocarditis poses a clinical challenge and has sparked renewed interest in the use of alternative approaches such as immunotherapy. Many similar adhesive proteins play a vital role in pathogenesis and are documented for their virulence and resistance not only in *E. faecalis* but also in other drug-resistant pathogens [13]. Most of these proteins are antigenic and IgG Fab fragments against the aggregation substance have conferred partial protection, as evidenced in studies on endocarditis [14]. Passive immunisation using monoclonal antibodies raised against the major component of pili has prevented the establishment of endocarditis [15]. The urgent need for cutting-edge therapeutic approaches to treat drug-resistant *E. faecalis* is evident. The development of vaccines is a crucial strategy for treating and preventing diseases caused by *E. faecalis*, as these vaccines must bind to immunological receptors. This is considered an important step to initiate any humoral and cell-mediated immune response in the host and the selection of dominant B-cell and T-cell epitopes is critical when constructing a vaccine.

The immune-informatic approach has successfully aided in the detection of promiscuous *EfbA* vaccine peptides in the present study by utilising various bioinformatics tools and databases within a single computer platform [16]. The molecular docking approach can model the interaction between a small molecule and a protein at the atomic level, characterising the behaviour of small molecules in the binding sites of target proteins, as well as elucidating fundamental processes. The Kolaskar & Tongaonkar antigenicity prediction method is employed to identify antigenic determinants on proteins. In order to address the global need to eradicate *E. faecalis* infections, this study aims to develop an in-silico multi-epitope peptide vaccine containing the most potent antigenic, non allergenic and non toxic epitopes from the selected immunogenic proteins. In vaccine peptide analysis, the proper selection of specific proteins is a crucial step and the present study focused on predicting epitopes from the adhesive protein *EfbA*. The location of the protein is essential in inducing an antibody response and the subcellular location, along with their non allergenic nature, makes the selected protein ideal for epitope predictions and further vaccine synthesis [17].

The secondary structure of the *EfbA* protein is also predicted, detailing the percentages of alpha helices, beta sheets, twists and coils, as the protein topology is influenced by its secondary structure. The SOPMA server serves the purpose of predicting the percentage of the secondary structure of the *EfbA* protein based on the amino acid sequence provided as input to the server. From 17 peptides, five were chosen based on antigenicity and stability analysis predictions. The epitopes IRAKGKNHK and LLLSAHPSY showed 100% similarity in the most favoured regions under Ramachandran validation. Using the Epidock server, MHC class II binders for T-cell epitopes were predicted and two epitopes were selected based on the maximum number of binders to assess their ability to elicit cellular immunity. The ability of HLA-DQ, HLA-DP, HLA-DR and TLR2 to present pathogenic peptides to activate T cells that secrete cytokines, as documented in earlier studies, was evaluated in the present investigation as well [17].

The docking interaction score between the epitope LLLSAHPSY and HLA-DP, DQ, DR and TLR4 shows a promising binding score with van der Waals interactions, conventional hydrogen bonds, carbon-hydrogen bonds, pi-sigma bonds, alkyl-pi bonds and pi-alkyl bonds.

This epitope has also demonstrated an antigenic peak in the B-cell epitope prediction tool. Based on these computational evaluations, the epitope LLLSAHPSY has been selected in the present study for further experimental validation in-vivo for its immunological memory and response.

Limitation(s)

The small sample size on which the data findings were based was not statistically analysed. The time period of the study was also limited and thus the frequency of the *EfbA* gene among clinical strains of *E. faecalis* cannot be substantiated. The in-silico analysis assisted in the prediction of novel epitope peptides; however, the need for further peptide synthesis and validation through preclinical trials is essential for a complete understanding of its immunological response in-vivo. Nonetheless, this study was the first of its kind to report and evaluate the immunodominant T-cell and B-cell dominant epitopes from the *EfbA* protein of *E. faecalis*.

CONCLUSION(S)

The present investigation documents the frequency of the *EfbA* virulent gene among clinical strains of *E. faecalis*. The strains are MDR, which warrants the need for frequent surveillance of these clinical strains in a dental healthcare setting. In-silico prediction identified the epitope LLLSAHPSY as a promising vaccine candidate from the *EfbA* of *E. faecalis*. The immune-informatics approach yielded promising results regarding its structure and validation in conjunction with antigenicity and stability predictions. However, the predicted epitope peptide requires further experimentation in animal models for its therapeutic application in treating infections caused by drug-resistant strains of *E. faecalis*. Based on the reported vaccine peptide, it is evident that constructing a vaccine against the EF3314 of *Enterococcus faecalis* is highly feasible.

Acknowledgements

The authors are thankful to the management of Saveetha Dental College for the opportunity to carry out the research work at the undergraduate level utilising the infrastructure of the institution.

Authors' contributions: DA- implemented the designed study: ASSG contributed for the conceptualisation and design, validation of the data obtained, manuscript drafting, editing and review; JVP contributed for the final validation and proof reading of the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

[1] Colodner R, Eliasberg T, Chazan B, Raz R. Clinical significance of bacteriuria with low colony counts of *Enterococcus* species. Eur J Clin Microbiol and Inf Dis. 2006;25:238-41.
[2] Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic susceptibility of periodontal *Enterococcus faecalis*. J Periodontol. 2013;84:1026-33.
[3] Chirouze C, Athan E, Alla F, Chu VH, Ralph Corey G, Selton-Suty C, et al. Enterococcal endocarditis in the beginning of the 21st century: Analysis from the International Collaboration on Endocarditis-Prospective Cohort Study. Clin Microbiol Infect. 2013;19:1140-47.
[4] Pinkston KL, Singh KV, Gao P, Wilganowski N, Robinson H, Ghosh S, et al. Targeting pili in enterococcal pathogenesis. Infect Immun. 2014;82(4):1540-47.
[5] Girija ASS, Ganesh PS. Functional biomes beyond the bacteriome in the oral ecosystem. Jap Dent Sci Rev. 2022;58:217-26.
[6] Kouidhi B, Zmantar T, Mahdouani K, Hentati H, Bakhrouf A. Antibiotic resistance and adhesion properties of oral Enterococci associated to dental caries. BMC Microbiol. 2011;11:155-59.
[7] Sogasu D, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterization and epitope-based vaccine predictions for *ompA* gene associated with biofilm formation in multidrug-resistant strains of *A.baumannii*. In Silico Pharmacology. 2021;9:01-09.
[8] Meiji Soe A, Urushibara N, Kawaguchiya M, Ohashi N, Hirose M, Kudo K. Antimicrobial resistance, virulence factors, and genotypes of *enterococcus faecalis* and *enterococcus faecium* clinical isolates in northern Japan: Identification of optrA in ST480 *E. faecalis*. Antibiotics. 2023;12(1):108.
[9] Clinical Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing, CLSI supplement, M100, USA, March 2021, Ed.31.
[10] Paganelli FL, Willems RJ, Leavis HL. Optimizing future treatment of enterococcal infections: Attacking the biofilm? Trends Microbiol. 2012;20(1):40-49.
[11] Pragash DS, Girija S, Sekar U, Rayapu V, Sheriff DS. Uropathogens and diabetes mellitus- a perspective. J Dent and Med Sci. 2017;16:29-32.
[12] Vijayashree Priyadharsini J, Smiline Girija AS, Arumugam P. An in-sight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile: An in-silico approach. Heliyon. 2018;4(12):e01051.
[13] Padmaja S, Smiline Girija AS, Vijayashree Priyadharsini J. Frequency of adhesive virulence factor fimH among the clinical isolates of *acinetobacter baumannii* in India J Pharmaceutical Res Int. 2020;16:12-17.
[14] Schlievert PM, Chuang-Smith ON, Peterson ML, Cook LCC, Dunny GM. *Enterococcus faecalis* endocarditis severity in rabbits is reduced by IgG Fabs interfering with aggregation substance. PLoS One. 2010;5(10):e13194.
[15] Ramarathinam SH, Ho BK, Dudek NL, Purcell AW. HLA class II immunopeptidomics reveals that co-inherited HLA-allotypes within an extended haplotype can improve proteome coverage for immunosurveillance. Proteomics. 2021;21:e2000160.
[16] Kazemian H, Pourmand MR, Siadat SD, Mahdavi M, Yazdi MH, Avakh Majelan P, et al. Molecular cloning and immunogenicity evaluation of PpiC, GelE, and VS87_01105 proteins of *enterococcus faecalis* as vaccine candidates. Iran Biomed J. 2019;23(5):344-53.
[17] Sette A, Rappuoli R. Reverse vaccinology: Developing vaccines in the era of genomics. Immunity. 2010;29:530-41.

PARTICULARS OF CONTRIBUTORS:
1. Undergraduate Student, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Institute (SIMATS), Chennai, Tamil Nadu, India.
2. Professor and Head, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Institute (SIMATS), Chennai, Tamil Nadu, India.
3. Associate Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Institute (SIMATS), Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. AS Smiline Girija,
Professor and Head, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, P.H. Road, Chennai, Tamil Nadu, India.
E-mail: smilinejames25@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 08, 2024
- Manual Googling: Oct 19, 2024
- iThenticate Software: Oct 21, 2024 (17%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **May 07, 2024**
Date of Peer Review: **Jun 10, 2024**
Date of Acceptance: **Oct 23, 2024**
Date of Publishing: **Jan 01, 2025**