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Original article

Vaccine candidates for cellulitis from *Staphylococcus aureus* and *Streptococcus pyogenes* – *In silico* approach

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ABSTRACT

Staphylococcus aureus and *Streptococcus pyogenes* are the causative agents of cellulitis, a bacterial skin infection. Cellulitis primarily affects the skin, and it later spreads to the other parts of the body, especially through the lymph nodes and bloodstream. Without proper medication, it becomes life-threatening. The main symptoms include inflammation, tenderness, tight and swollen skin, and fever. Vaccines using epitopes as antigens trigger quick immune responses; in addition, they are cost effective. These antigens are derived from bacterial proteins. In this study, virulence factors from membrane proteins such as sak, tst, isdA, clfB, and can from*S. aureus* (Fig. 1) and SPy, scpA, and hylp1 from *S. pyogenes* (Fig. 2) were selected for predicting the vaccine epitopes. VaxiJen server was used to evaluate the antigenicity of the selected proteins; BCPred, AAPPred, and ABCpred were used for B-cell epitope prediction, while ProPred and ProPred I were used for T-cell epitope prediction. MHCPred was used for the selected alleles, DRB1*0101 and DRB1*0401. Pepitope server was used for epitope mapping of the selected peptides. Epitopes that are common among those from BCPred, AAPPred, and ABCpred were selected: LKYGPKFDK (tst) and MTFDDKNGK (cna) from *S. aureus* and YTNSDKGGS (SPy), FKIEPDTTV (SPy), MTPSERLDL (scpA), VKTDDQQDK (scpA), and LKFKPAATV (hylp1) from *S. pyogenes*. Among them, YTNSDKGGS, MTPSERLDL, and MTFDDKNGK, were identified as suitable vaccine candidates for eliciting immune responses. These results of this study can be used to create a peptide-based vaccine for preventing cellulitis.

1. Introduction

Skin is the largest organ in the body with multiple functions; its breakdown leads toseveral diseases (Tortora and Grabowski, 1993). The global burden of diseases estimated in 2013associated 1.79% of skin disorders withsignificant morbidity and mortality; the numbers were especially high for cellulitis (Karimkhani et al. 2017). Considering the disability-adjusted life years, skin illnesses contributed to 1.79% of the worldwide burden of disease, among the 306 diseases (DALY's). Skin and soft tissue infections are majorly caused by bacteria, with skin inflammation (Karimkhani et al. 2017).

Cellulitis is a form of bacterial skin infection that can damage the dermis. It is caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, which cause inflammatory immune responses (Dennis and Bryant, 2016). These bacteria are present on the skin, and when the skin in damaged, they enter in through the damaged regions resulting in infection of the skin. The disease-causing bacteria are more virulent during the winter months in cold countries (Olafsdottir et al. 2015). The main symptoms of such infections include skin redness, skin colonizing, skin maceration, and redness

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of the skin within a few days after infection. The entrance point may not be obvious, and the breach can include modification in the superficial skin or could manifest as more intrusive infections depending on the type of bacteria. More than 95% of infections occur below the knee (Vary and Connor, 2014). Diabetic patients and elderly people are majorly affected by this disease (Karimkhani et al. 2017). *S. aureus* is overrepresented in the skin microbiome, and the local microbiome and immunity influence the relationship between the microbiome and cellulitis (Christensen and Bruggemann, 2014).

"Virulence" is an organism's capacity to infect its host and produce sickness. Virulence factors are components that help the bacteria establish an infection in the host at the cellular level. These elements either have a secretory, membrane-related, or cytosolic origin (Aditya Kumar et al., 2017). Virulence factors have contributed to the discovery of significant virulence traits in microbes, considerably advancing our understanding of microbial pathogenesis (Arturo and Pirofski, 2009).

When the pathogens enter into the break in the skin, they cause deep dermal and subcutaneous infection and cutaneous barrier disruption. Low surface pH, low temperature of the skin surface, and the occurrence of common microbes on the skin surface reduce colonization by pathogens (Raff and Kroshinsky, 2016). Heavy neutrophil infiltration around the blood vessels and dermal edema lymphatic dilation are important histological features of this disease (Burian et al., 2021). The primary strategy to combat cellulitis is vaccination. Peptide-based vaccines are novel and promising for the treatment of mild to chronic cellulitis (Wang and Walfield, 2005).

The UK National Health Service (NHS) conducted an analysis for cellulites diseases using the secure Anonymized information linkage databank, which compiles population-scale, individual-level anonymized linked data from a variety of sources, including 80% of primary care general practices in Wales (Humphreys et al., 2023) The number of general practice visits and in-patient stays were tracked for all patients linked to the pertinent codes in primary care settings for the last twenty years. The Welsh and UK NHS would experience significant financial savings through initiatives to assist patients and healthcare providers in identifying early indicators and dangers of cellulitis, enhance the accuracy of the initial diagnosis, avoid cellulitis recurrence, and improve evidence-based treatment pathways (Humphreys et al., 2023). In response to these discoveries, Wales has created the ground-breaking National Lymphoedema Cellulitis Improvement Program, which offers a proactive approach to cellulitis management (Humphreys et al., 2023).

Therefore, this study aimed to identify possible B-cell and T-cell epitopes from the pathogenic membrane proteins that could stimulate immune responses, both cellular and humoral (Hajighahramani et al. 2017).

2. Materials and methods

2.1. Selection of virulence proteins

Virulence factors for cellulitis were selected from the membrane proteins of *S. aureus* and *S. pyogenes*. Five proteins were selected from *S. aureus* – sak, tst, isdA, clfB, and cna (Fig. 1) and three proteins from *S. pyogenes* – SPy, scpA, and hylpL (Fig. 2). The antigenic properties of these proteins were predicted.

2.2. Retrieval of membrane protein sequences

The amino acid sequences of sak (P68802), tst (p06886), isdA (Q7A655), clfB (Q6GDH2), cna (Q53654), SPy (Q9A1S2), scpA (B6ETQ5), and hylpL (Q9A0M7) were retrieved from UniprotKB database.

2.3. Prediction of antigenicity of membrane proteins

The VaxiJen 2.0 server was used to determine the immunogenic characteristics of the recovered sequences for improving the prediction accuracy and reducing the false positives (Doytchinova and Flower, 2007). The sequences with a VaxiJen score of $\geq$ 0.7 were chosen.

2.4. B-cell epitope prediction

B-cell epitopes were predicted from sequences with VaxiJen score $\geq$ 0.7 using BCPred and AAPPred. This resulted in the identification of 20-mers using ABCpred, which resulted in 16-mer antigenic sequences (https://webs.iiitd.edu.in/raghava/propred1/) (Singh and Raghava, 2003).

2.5. T-cell epitope prediction

The chosen B-cell epitope antigens were used for T-cell epitope prediction using ProPred I based on the binding affinity with Major Histocompatibility Complex (MHC) classI, for 51 alleles (Singh and Raghava, 2003) and with MHC II, for 47 alleles (Singh and Raghava, 2001). Selected 9-mer epitopes with affinity to both MHC I and MHC II were selected. (https://webs.iiitd.edu.in/raghava/propred/).

2.6. MHCPred prediction

The prediction was made using MHCPred, based on MHC binding affinity. The IC_{50} values of the specific alleles, DRB1*0101 and DRB1*0401, such as predicted logIC_{50}, predicted IC_{50}, and confidence of prediction values were calculated. The sequences in FASTA format were evaluated using MHCPred (Guan et al., 2003) (https://www.ddgpharmfac.net/mhcpred/MHCPred/).

2.7. Target protein from protein data bank

The 3D structures of the selected 8 membrane proteins: sak (1C77), tst (2QIL), isdA (3QZO), clfB (4F24), and cna (1D2O) from *S. aureus* and SPy (3B2M), scpA (3EIF), and hylpL (3EKA) from *S. pyogenes* were retrieved from protein data bank (PDB).

2.8. Pepitope server

Epitopes were computationally predicted using the Pepitope server, and the epitope localization on the target protein was visualized. (Mayrose et al. 2007). To detect the epitope on the protein, epitope mapping was performed using PepSurf (Mayroseet al., 2006) and Mapitope (Bublil et al., 2007) (https://pepitope.tau.ac.il/).

3. Results

3.1. Virulence-associated membrane proteins and the characterization of their antigenic properties

sak, tst, isdA, clfB, cna, SPy, scpA, and hylpL were studied for their antigenic potential based on the criteria of VaxiJen score of $\geq$ 0.7 (Table1).

3.2. B-cell epitope prediction

The number of B-cell epitopes predicted using BCPred, AAPpred, and ABCpred for the selected proteins, sak, tst, isdA, clfB, and cna

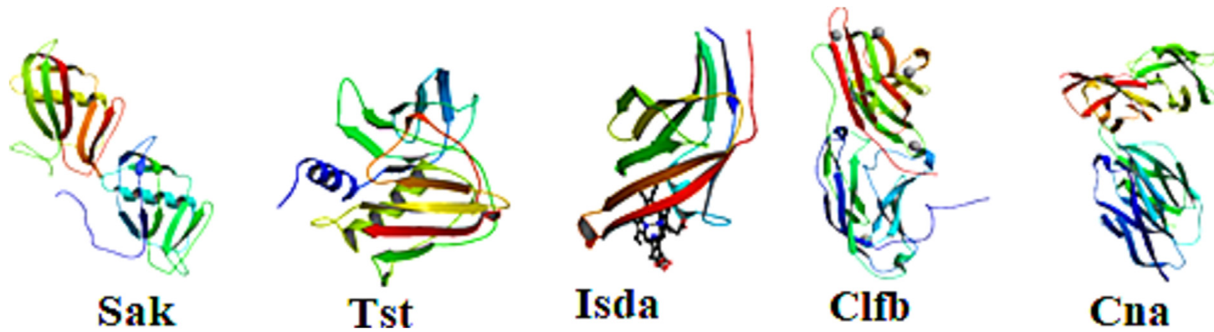

Fig. 1. 3D structures of selected *S. aureus* membrane proteins.

Fig. 2. 3D structures of selected *S. pyogenes* membrane proteins.

Table 1
B-cell epitopes predicted using BCPred, AAPPred, and ABCpred.

S. No	Membrane proteins	Uniprot ID	VaxiJen score	Number of identified B-cell epitopes using BCPred, AAPPred, and ABCpred	Number of accepted B-cell epitopes from BCPred, AAPPred, and ABCpred
Staphylococcus aureus					
1	Sak	P68802	0.7026	4 + 3 + 17	3 + 0 + 8
2	Tst	P06886	0.8659	5 + 6 + 27	3 + 4 + 21
3	isdA	Q2FZE9	0.7313	10 + 10 + 45	7 + 5 + 25
4	clfB	A0A0E1AQ60	1.1204	24 + 22 + 116	19 + 19 + 98
5	Can	A0A0E1AM77	0.7300	24 + 25 + 94	15 + 16 + 54
Streptococcus pyogenes					
1	Spy	Q9A1S2	0.8431	9 + 9 + 41	8 + 8 + 26
2	scpA	P15926	0.5868	33 + 31 + 127	20 + 18 + 52
3	hylpL	Q9A0M7	0.8171	7 + 9 + 40	7 + 5 + 26

from *S. aureus* were 24, 38, 65, 162, and 143, but the accepted B-cell epitopes were 11, 28, 37, 136, and 85. The epitopes were narrowed down based on their VaxiJen antigenic score. For *S. pyogenes*, the number of epitopes identified from SPy, scpa, and hylpL were 59, 191, and 56 whereas the accepted B-cell epitopes were 42, 90, and 38 (Table 1). BCPred and AAPPred identified 20-amino acid B-cell epitopes, while ABCpred predicted epitopes with 16 amino acids (Supplementary Table 1).

3.3. T-cell Epitope prediction

To identify T cell epitopes, the chosen B-cell epitopes were evaluated using ProPred1 and Propred with the standard parameters. The VaxiJen score, MHCPred score, and the surface localization of the epitope in the theoretical model were used to identify epitopes. DRB1*0101 and DRB1*0401 are the MHC class I and II alleles, respectively, to which the common epitopes most frequentlybind. T-cell epitopes with the potential to interact with MHC class I and II molecules were predicted using ProPred1, for the MHC class I-binding regions of the antigens from 47 alleles and using ProPred, for the MHC class II-binding regions of the antigens from 51 alleles, from the B-cell VaxiJen antigenic epitopes. The binding regions identified using both the servers were used for further analysis of vaccine candidates. Finally, 9-mer epitopes predicted using both ProPred and ProPred 1 were shortlisted (Table 2) for the next step.

The epitopes identified using BCPred, AAPPred, and ABCpred (Table 2) were selected for screening using Pepitope.

The final epitopes predicted from *S. aureus* are LKYGPKFDK and MTFDDKNGK and from *S. pyogenes* are YTNSDKGGS, FKIEPDTTV, MTPSERLDL, VKTDDQQDK, and LKFKPAATV. Pepitope, which identifies the best epitope cluster, was used to find the epitope clusters on the protein's outer membrane (Table 3).

3.4. Pepitope cluster

Among the 58 epitopes identified from *S. aureus*, only the two best clusters, LKYGPKFDK and MTFDDKNGK, were present on the outer surface of the protein (Fig. 3).

Table 2
T-cell epitope prediction.

S. No	Protein name	B-cell epitopes	Simultaneous T-cell epitope	Number of selected alleles in MHC I + MHC II	Score for T-cell Epitope VaxiJen
Staphylococcus aureus					
1	sak	KIEVTYYDKNKKKEETKSFP	YYDKNKKKE	5 + 3	0.7702
		–	–	–	–
		AKIEVTYYDKNKKKEE	YYDKNKKKE	1 + 3	0.7702
2	tst	GLYRSSDKTGGYWKITMNDGVHGKDSPLKYGPKFDKKQLA	YRSSDKTGG	3 + 11	2.37811.3422
			LKYGPKFDK	2 + 11	
		VKVHGKDSPLKYGPKFDKKQTQIHGLYRSSDKTGGYWKIT	**LKYGPKFDK**	2 + 11	1.3422
			YRSSDKTGG	3 + 11	2.3781
		DSPLKYGPKFDKKQLA	**LKYGPKFDK**	2 + 11	1.3422
		DGSISLIIFPSPYYSP	LIIFPSPYY	9 + 21	1.3917
		EGTYIHFQISGVTNTE	FQISGVTNT	6 + 18	0.8110
		PTPIELPLKVKVHGKD	YIHFQISGV	5 + 8	1.1966
		YWKITMNDGSTYQSDL	LPLKVKVHG	2 + 10	1.1893
		NFFIVSPLLLATTATD	WKITMNDGS	3 + 5	1.7053
		ESVMNKKLLMNFFIVSSMRIKNTDGSISLIIF	FFIVSPLLL	12 + 29	0.269
			FIVSPLLLA	5 + 18	1.26152.14782.3375
			LLMNFFIVSMRIKNTDGS	1 + 10	
				3 + 38	
3	isdA	AKPNNVKPVQPKPAQPKTPT	VKPVQPKPAVQPKPAQPK	2 + 7	1.09471.3176
				3 + 4	
		VEPGYKSLTTKVHIVVPQINKYQSEQRSSAMKKITMGTAS	VHIVVPQIN	1 + 6	0.8454
			YKSLTTKVH	3 + 24	0.7420
			YQSEQRSSA	7 + 1	1.0402
		LVYIGADSQQVNAATE	YIGADSQQV	5 + 7	0.8840
		NSKYQSEQRSSAMKKI	YQSEQRSSA	7 + 1	1.0402
		QELATTVVNDNKKADT	VVNDNKKAD	2 + 11	1.0792
		KVHIVVPQINYNHRYT	VHIVVPQIN	1 + 6	0.8454
		EPGYKSLTTKVHIVVPSSTAPHYLCCCSARES	YKSLTTKVHYLCCCSARE	3 + 24	0.74200.7326
				3 + 3	
4	clfB	ANSQVDNKTTNDANNIATNSGNTWVVYIKGYQDKIEESSGK	VDNKTTNDAVYIKGYQDK	4 + 2	1.91520.7424
				1 + 2	
		VPQEANSQVDNKTTNDANNI	VDNKTTNDA	4 + 2	1.9152
		PNPNQYKVEFNTPDDQ	YKVEFNTPD	2 + 4	1.1903
		TDYVNTKDDVKATLTM	YVNTKDDVK	5 + 2	1.0564
		TVGIDSGTTVYPHQAG	VGIDSGTTV	8 + 8	1.0491
		TFKITVPKELNLNGVT	FKITVPKEL	11 + 14	0.9177
		QAVNTSAPRMRAFSLANPENFEDVTNSVNITF	VNTSAPRMRVTNSVNITF	2 + 2	1.50730.9582
				8 + 3	
5	cna	YVSKDITIKDQIQGGQQLDLVKMTFDDKNGKIQNGDTIKV	YVSKDITIK	5 + 9	0.99921.46721.8150
			IQNGDTIKV	9 + 12	
			MTFDDKNGK	5 + 8	
		LPKYDEGKKIEYTVTEDHVK	IEYTVTEDH	1 + 2	1.1265
		PGSKITVDNTKNTIDVTIPQ	YVSKDITIK	5 + 2	1.4125
		FAEFEVQGRNLTQTNTSDDK	ITVDNTKNT	3 + 4	0.8680
		NEKRYVSKDITIKDQIQGGQVKMTFDDKNGKIQNGDTIKV	VQGRNLTQT	5 + 1	1.3175
			YTVTEDHVK	5 + 9	0.9992
			MTFDDKNGK	6 + 8	0.4141
		QKEIEIKTDANGIANI	IKTDANGIA	2 + 3	1.3925
		GKKIEYTVTEDHVKDY	IEYTVTEDH	1 + 2	1.1265
		ARDISSTNVTDLTVSP	YTVTEDHVK	5 + 2	1.4125
		GGKTTVKMTFDDKNGK	ISSTNVTDL	12 + 2	0.7634
		NGKIQNGDTIKVAWPT	MTFDDKNGK	5 + 8	1.8150
		EGTQKVKPTIYFKLYK	IQNGDTIKV	9 + 12	1.4672
		PTIYFKLYKQDDNQNT	VKPTIYFKL	14 + 6	1.6698
		TVKIEGYSKTVSLTVK	YKQDDNQNT	2 + 3	1.6119
		EINCNASSTAPHYLCC	YSKTVSLTV	9 + 3	0.7988
		APHYLCCCSARESSSPKMTFDDKNGKIQNGDT	IEGYSKTVS	3 + 6	0.9612
			INCNASSTA	2 + 1	1.6269
			YLCCCSARE	3 + 3	0.73261.8150
			MTFDDKNGK	5 + 8	
Streptococcus pyogenes					
6	SPy	YVVTEDDYKSEKYTTNVEVSMTKVTYTNSDKGGSNTKTAEPNTDFTFKIEPDTTVNEDGN	YVVTEDDYK	6 + 2	1.12172.24302.3066
			YTNSDKGGS	2 + 6	
			FKIEPDTTV	6 + 3	
		YTNSDKGGSNTKTAEFDFSE	**YTNSDKGGS**	2 + 6	2.2430
		IPNTDFTFKIEPDTTVNEDGFGLTLKANQYYKASEKVMIE	**FKIEPDTTV**	6 + 3	2.3066
			FGLTLKANQLTLKANQYY	2 + 10	1.33690.8411
				8 + 1	
		TFKIEPDTTVNEDGNK	**FKIEPDTTV**	6 + 3	2.3066
		YVVTEDDYKSEKYTTN	YVVTEDDYK	6 + 2	1.1217

Table 2 (*continued*)

S. No	Protein name	B-cell epitopes	Simultaneous T-cell epitope	Number of selected alleles in MHC I + MHC II	Score for T-cell Epitope VaxiJen
		VSPQDGAVKNIAGNST	VKNIAGNST	1 + 3	0.7509
		TVVNGAKLTVTKNLDL	VVNGAKLTV	5 + 28	0.8035
		TTVHGETVVNGAKLTV	LTVTKNLDL	12 + 4	0.8892
		PMTKVTYTNSDKGGSN	VVNGAKLTV	5 + 28	0.8035
		PGVYYYKVTEEKIDKV	**YTNSDKGGS**	2 + 6	2.2430
		KVPIQFKNSLDSTTLT	YYYKVTEEK	3 + 8	1.1890
		DFEVPTGVAMTVAPYI	IQFKNSLDS	2 + 37	1.1565
		AVKNIAGNSTEQETSTSKDFNFGLTLKANQYY	VPIQFKNSL	23 + 7	0.7694
			VAMTVAPYI	13 + 4	1.0109
			VKNIAGNSTFNFGLTLKA	1 + 3	0.75091.7373
				6 + 21	
7	scpA	DAKKASAATMYVTDKDNTSS	YVTDKDNTS	1 + 10	0.9313
		LQKQYETQYPDMTPSERLDL	**MTPSERLDL**	7 + 4	1.1479
		AYANRGMKEDDFKDVKGKIA	YPDMTPSER	7 + 2	1.0347
		KDQLDGDGLQFYALKNNFTA	YANRGMKED	1 + 1	0.8781
		TAMVKTDDQQDKEMPVLSTNRGDIDFKDKVANAKKAGAVG	YALKNNFTA	12 + 6	0.8630
			VKTDDQQDKFKDKVANAK	1 + 6	1.83700.7177
				2 + 1	
		TAMVKTDDQQDKEMPVLSTN	**VKTDDQQDK**	1 + 6	1.8370
		QYETQYPDMTPSERLDLAKKRTLEKRSSKRALATKASTRD	**MTPSERLDL**	7 + 4	1.1479
			YPDMTPSERLEKRSSKRA	7 + 2	1.03471.7010
				6 + 1	
		YETQYPDMTPSERLDL	**MTPSERLDL**	7 + 4	1.1479
		TAMVKTDDQQDKEMPV	YPDMTPSER	7 + 2	1.0347
		YIHRHANGEPYAAISP	**VKTDDQQDK**	1 + 6	1.8370
		GSSYYHEANSDAKDQL	YIHRHANGE	1 + 4	1.2629
		SRTLEKRSSKRALATK	YYHEANSDA	4 + 4	1.0072
		YVTDKDNTSSKVHLNN	LEKRSSKRA	6 + 1	1.7010
		ANNKYAKLSGTSMSAP	YVTDKDNTS	1 + 10	0.9313
		RGDIDFKDKVANAKKA	YAKLSGTSM	10 + 1	0.7302
		AYANRGMKEDDFKDVK	FKDKVANAK	2 + 1	0.7177
		VQTDKVDGKHFALAPK	YANRGMKED	1 + 1	0.8781
		SRTLEKRSSKRALATK	VQTDKVDGK	4 + 10	2.1472
		YVTDKDNTSSKVHLNN	LEKRSSKRA	6 + 1	1.7010
		ANNKYAKLSGTSMSAP	YVTDKDNTS	1 + 10	0.9313
		RGDIDFKDKVANAKKA	YAKLSGTSM	10 + 1	0.7302
		AYANRGMKEDDFKDVK	FKDKVANAK	2 + 1	0.7177
		VQTDKVDGKHFALAPK	YANRGMKED	1 + 1	0.8781
		KVVANGTYTYRVRYTP	VQTDKVDGK	4 + 10	2.1472
		ELYYQATVQTDKVDGKLVAHIFKTKRQKETKK	YTYRVRYTP	3 + 4	1.1699
			VQTDKVDGK	4 + 4	2.1472
			YQATVQTDKFKTKRQKET	6 + 4	0.85111.1338
				2 + 2	
8	hylpL	NITSGNENGSAMQLRGSEKANLKGGVMTGQLKFKPAATVA	MQLRGSEKA	3 + 4	1.20061.7727
			LKFKPAATV	6 + 12	
		NGAGTAAQGIYINSTSGTTGMTGQLKFKPAATVAYSSSTG	IYINSTSGT	3 + 12	1.5134
			YINSTSGTT	3 + 2	1.72471.7727
			LKFKPAATV	6 + 12	
		GGVMTGQLKFKPAATV	**LKFKPAATV**	6 + 12	1.7727
		AVNIDLSSTRGAGVVV	VNIDLSSTR	2 + 12	1.7435
		GNLKLKDPTANDHAAT	IDLSSTRGA	6 + 1	1.5063
		DYKGTTNAVNIAMRQP	LKLKDPTAN	2 + 5	1.2230
		AQGIYINSTSGTTGKL	YKGTTNAVN	3 + 4	0.8176
		GSAMQLRGSEKALGTLYNLLTNKPNIDGLATK	IYINSTSGT	3 + 12	1.5134
			YINSTSGTT	3 + 2	1.7247
			MQLRGSEKAYNLLTNKPN	3 + 4	1.20060.9157
				2 + 7	

Five best epitopes of *S. pyogenes* were shortlisted from among the 64 epitopes. Among them, FKIEPDTTV, MTPSERLDL, and LKFKPAATV (Fig. 4) were present on the outer surface of the protein. The red regions are the epitopes present on the outer surface of the protein.

IC_{50} values of 0.01 to 5000 nM indicate that the sequence is eligible; < 5000 nM indicates low affinity, < 500 nM indicates intermediate affinity, and < 50 nMindicates high affinity. The epitopes were selected as vaccine candidates based on the IC_{50} values for both alleles, DRB1*0101 and DRB1*0401 (Table 4), the most-prevalent MHC alleles in mammals. Further experimental validation of the vaccine candidates is necessary. Among the two epitopes from *S. aureus*, MTFDDKNGK exhibited an intermediate binding affinity for DRB1*0101 with a IC_{50} value of 84.33, which is slightly higher than the threshold value of 50 nM; it had a low affinity for DRB1*0401. LKYGPKFDK exhibited low affinity for both alleles. In case of *S. pyogenes* epitopes, both YTNSDKGGS and MTPSERLDL had a high binding affinity for DRB1*0101; the former had an intermediate and the latter had a low affinity for DRB1*0401. FKIEPDTTV had intermediate binding affinity for

Table 3
Epitope cluster prediction using Pepitope.

S.No	Protein name	Epitope/s
Staphylococcus aureus		
1.	tst	LKYGPKFDK
2.	cna	MTFDDKNGK
Streptococcus pyogenes		
1.	Spy	YTNSDKGGSFKIEPDTTV
2.	scpA	MTPSERLDLVKTDDQQDK
3.	hylpL	LKFKPAATV

DRB1*0101 and low binding affinity for DRB1*0401. Both VKTDDQQDK and LKFKPAATV showed low affinity for both alleles.

4. Discussion

The two most common bacteria that cause cellulitis are *S. aureus* and *S. pyogenes* (Bennett et al., 2010); they are frequently encountered and clinically challenging (Sullivan and Barra, 2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) in the population increased skin and soft tissue infections from 1993 to 2005 (Pallinet al., 2008). In a recent studyincluding40 locations and 9 countries encompassing 7477 chronic oedema patients, 16% had experienced cellulitis in the previous 12 months with a prevalence of 37% (Burian et al., 2021).

To avoid the risk factors associated with cellulitis, it is essential to identify an effective vaccine; this strategy is widely used to tackle the incidence of diseases with high risk (Oscherwitz, 2016). A vaccine for *S. aureus* against bacteremia and pneumonia is available (Adhikari et al. 2012). This study aimed to predict a vaccine for cellulitis.

Both the humoral response involving B-cells and cell-mediated immunity involvingT-cells are important for adaptive immunity. Pathogens as a whole are not recognized by B- and T-cells; however, molecular components known as antigens are recognized (Paul et al., 2015). Epitopes as vaccine candidates have no undesirable effect; therefore, they are preferred over whole protein vaccines (Gallimore et al., 1998). The research of Correia et al. (2014) supports the epitope-focused vaccine design employed in this study.

Locating possible B- and T-cell epitopes using computational techniques, such as immunoinformatics reduces the time and lowers the cost involved in pathogen gene product investigation in the laboratory. (Davies and Flower, 2007). Mondal et al. (2019) showed that the virulence factor and outer membrane proteins are promising candidates for designing epitopic vaccines. Therefore, the virulence factor-based membrane proteins, such as sak, tst, isdA, clfB, and can from *S. aureus* and SPy, scpA, and hylp1 from *S. pyogenes* were selected using VaxiJen server with a VaxiJen antigenicity cut-off score of $\geq$ 0.7, with 70–89% prediction accuracy (Verjovski-Almeida et al., 2003). According to Tong et al., (2009), the majority of investigations use linear epitopes, similar to that predicted in this study, because these B-cell epitopes interact with B-cell receptors to form antigenic determinants on the surface of pathogens. Chen et al. (2007) suggested that utilizing the three tools, BCPred, AAPPred, and ABCpred will increase the accuracy of epitope prediction. B-cells provide long-term protection against pathogens and harmful molecules (Jespersen et al., 2019); therefore, 20-mer B-cell epitopes (Singh et al., 2013) were predicted using BCPred and AAPred, and 16 *mer*-epitopes (EL-Manzalawyet al., 2008) were predicted using ABCpred. The number of accepted B-cell epitopes were 11, 28, 37, 136, and 85 from sak, tst, isdA, clfB, and cna of *S. aureus* and 42, 90, and 38 fromSPy, scpa, and hylpL of *S. pyogens.*

A vaccine delivers antigenic substances that trigger immune reactions to provide an efficient degree of protection against a disease (Kreikemeyeret al., 2017). Our earlier research concentrated on identifying conserved epitopes in several *S. pyogenes* membrane proteins. Therefore, it is possible to develop conserved peptide-based streptococcal vaccines against human illnesses (Ebrahimi and Mohabatkar, 2018). Clinical application of these peptide-based vaccines against *S. pyogenes* requires further studies and experimental validation.

Numerous *S. aureus* strains are resistant to common clinical drugs, including vancomycin (Howdenet al., 2010), daptomycin (Fowler et al., 2006), mupirocin (Cadillaet al., 2011), and linezolid (Locke et al., 2009). There is ongoing research for developing novel molecules, especially vaccines, to fight these infections. An important strategy to combat *S. aureus* is creating potent monoclonal

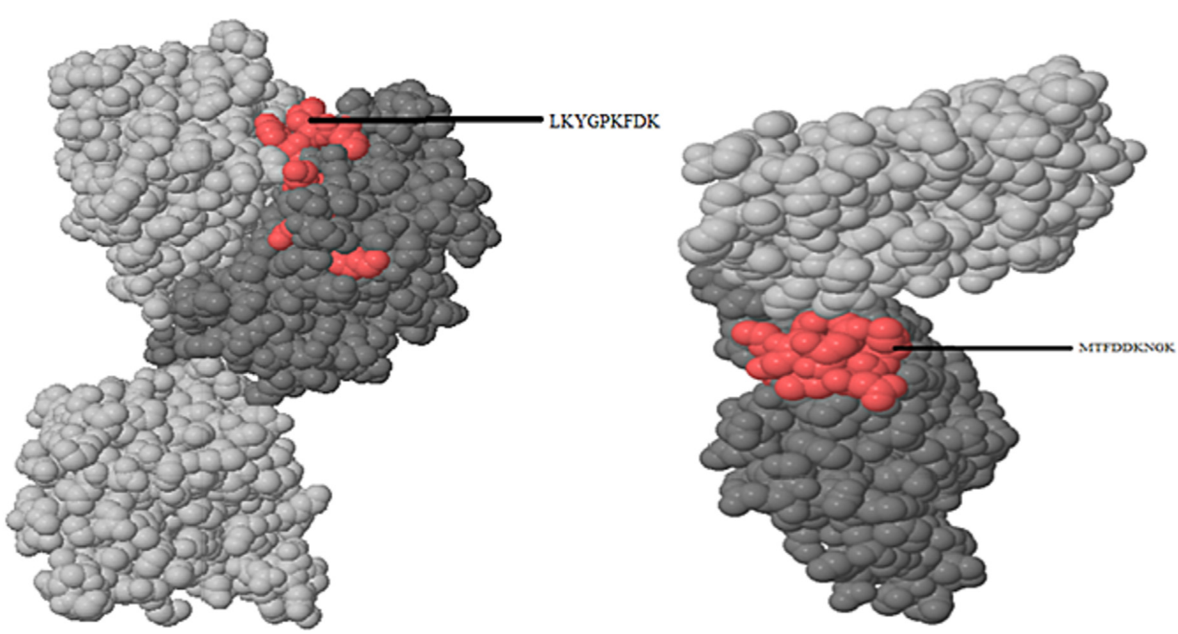

Fig. 3. Epitopes of *S. aureus* predicted using Pepitope.

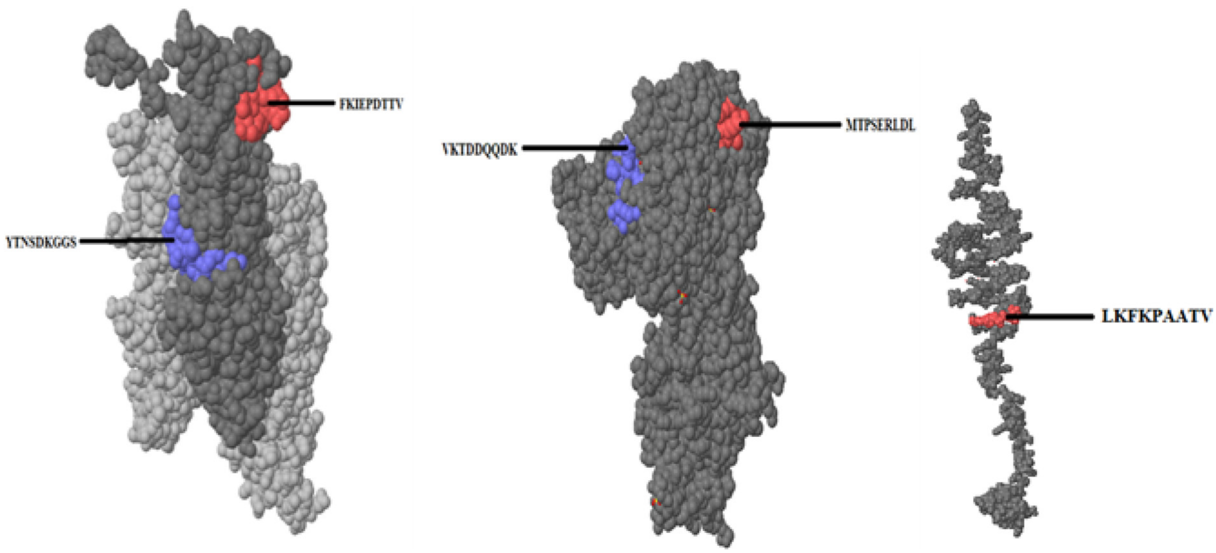

Fig. 4. Epitopes of *S. pyogenes* predicted using Pepitope.

Table 4
Validation of epitopes from membrane proteins and MHCPred values.

S.No	Protein name	T-cell epitopes	IC_{50}values of DRB1*0101 (MHCPred)	IC_{50} values of DRB1*0401 (MHCPred)
Staphylococcus aureus				
1.	tst	LKYGPKFDK	944.06	2697.74
2.	cna	MTFDDKNGK	84.33	1729.82
Streptococcus pyogenes				
1.	SPy	YTNSDKGGSFKIEPDTTV	33.81268.53	597.04672.98
2.	scpA	MTPSERLDLVKTDDQQDK	29.85620.87	1945.36571.48
3.	hylpL	LKFKPAATV	706.32	3118.89

antibodies and vaccines. However, traditional inactivated vaccines and capsular polysaccharide vaccines are not the best options for providing immunoprotection. Therefore, the B-cell epitope, [272]-GYTEDEIV[279] is promising for eliciting a potent immunoprotection against *S. aureus* infection.

Predicting T-cell epitopes requires the identification of MHC-binding peptides because T-cell recognition of antigenic peptides requires their association with MHC (Tomar and De, 2010). Short peptides of 9–11 amino acids can bind to MHC I; therefore, the 9-mer T-cell epitopes, which bind to MHC I and MHC II with high intensity were selected for predicting the affinity for the human MHC alleles, DRB1*0101 and DRB1*0401. The frequency of the two selected alleles, DRB1*0101 and DRB1*0401 within MHC class-II ranges between 20 and 50%. They are among the most common HLA-DR alleles; therefore, these two HLA molecules were chosen during MHCPred analysis (Panigada et al., 2002; Saha et al., 2017). It is essential for a peptide to bind to more than one HLA allele to be considered as a potential peptide for vaccine development. All five epitopes assessed in this study, two from *S. aureus* and three from *S. pyogenes*, were present on the surface, as indicated by the results of epitope mapping.

5. Conclusion

Cellulitis is a typical bacterial skin infection that causes pain, swelling, and redness in the infected region. We aimed to develop a new vaccine candidate from virulence-associated membrane proteins, through epitope identification, that is selective against the pathogens and invokes effective immune responses against this skin disease. Several steps of screening were performed to identify the appropriate vaccine candidates. The epitope, YTNSDKGGS, identified from fctA of *S. pyogenes* is promising as a prospective vaccine candidate, considering the binding affinity and high antigenic values. In addition, the epitopes, MTPSERLDL from scpA of *S. pyogenes* and MTFDDKNGK from cna of *S. aureus*, are present on the outer surface of their respective proteins. They exhibited better affinity for the two MHC alleles; therefore, they are promising as vaccine candidates as well. Future studies should focus on *in vitro* and *in vivo* evaluations focused onverifying the effectiveness of these vaccine candidates.

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Ethical statement

This research work does not contain any human/animal sample.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102917.

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