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Prediction of B-Cell epitopes on CbpA, PspA and PhtD protein of Streptococcus pneumonia using Immunoinformatics approach

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Abstract

Pneumococcal infections range from simple respiratory tract mucosal infections as otitis media and sinusitis to more severe diseases including pneumonia, septicemia and meningitis. There is a need for enhanced vaccines or drugs as a result of its epidemic outbreak and the lack of potential medication. B-cell mediated adaptive immunity is capable of developing pathogen-specifc memory that confers immunological protection. Therefore, in this study, the envelope protein of the Zika virus was retrieved from the NCBI protein database. The ABCpred and BepiPred software were used to discover linear B-cell epitopes on envelope protein. Conformational B-cell epitopes on envelope protein were identifed using SEPPA 3.0 and Ellipro tools. Predicted B-cell epitopes were evaluated for allergenicity, toxicity, and antigenicity. Consensus linear B-cell epitopes, Choline binding protein A (344-359KKKAEDQKEEDRRNYP, 281-296DAKEQGKPKGRAKRGV, 191-206EKKAKDQKEEDRRNYP, 314-329DSSVGEETLPSPSLKP), Pneumococcal surface protein A (68-83AEDAQKKYEDDQKRTE, 41-56SSLEKKYEEAKAKADT) and Pneumococcal histidine triad protein D (593-608TDHQDSGNTEAKGAEA, 587-602GLTPPSTDHQDSGNTE, 38-53RVSYIDGDQAGQKAEN)
were identifed using ABCpred and BepiPred tools. SEPPA 3.0 and Ellipro tools predicted consensus conformational Choline binding protein A (93-97STKKR), Pneumococcal surface protein A (117-125RSKYKSDAE, 491-493PKP, 326-331ADPEDD, 406-413GADSEDDT) and
Pneumococcal histidine triad protein D (24-26LGR, 29-31AGQ, 266-272HQNQGEN) as a component of B-cell epitopes. These predicted linear and conformational B-cell epitopes will help in designing peptide vaccines that will activate the humoral response. However, in-vitro and in-vivo laboratory experimental confrmations are still needed to prove the application's feasibility.

Keywords: Immunoinformatics, CbpA, PspA, PhtD, B-cell epitopes, Streptococcus pneumonia

Introduction

Streptococcus pneumonia (pneumococcus) is a Gram-positive, extracellular bacterial pathogen that causes significant morbidity and mortality each year, especially in children, elderly, and those with compromised immune systems (Centers for Disease Control and Prevention, 2019). Pneumococcal infections range from simple respiratory tract mucosal infections as otitis media and sinusitis to more severe diseases including pneumonia, septicemia and meningitis. Worldwide, there have been over 14 million cases of severe pneumococcal diseases, and each year, about 1.6 million people die from pneumococcal infection (Black et al., 2010). In 2019, China demonstrated that pneumococci were resistant to clindamycin, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole in 95.8%, 95.2%, 93.6%, and 66.7% of cases, respectively (Wang et al., 2019). Furthermore, current pneumococcal vaccines are neither 100% efficacious nor protective against all pneumococcal serotypes. In the production of pneumococcal vaccines, surface antigens, such as immunogenic proteins and polysaccharides, are utilized (Bridy-Pappas *et al.,* 2005). The 7, 10, or 13-valent pneumococcal conjugate vaccines and the 23-valent Streptococcus pneumoniae polysaccharide vaccine (PPV) induce serotype-specific immunity (Pichichero *et al.,* 2016). Pneumococcal conjugate vaccine PCV13 was released in 2010, although it only covers 13 serotypes of pneumococci out of 95. Although the PCV13 vaccine has benefitted older people by preventing the route of bacterial transmission and greatly reduced pneumococcal infection in children (Simonsen et al., 2011). Unfortunately, the effectiveness rate of a PCV13 immunization plan against IPD has declined due to community substitution of vaccine serotypes with non-vaccine serotypes. As a result, it is necessary to identify novel vaccine candidates, particularly a protein/peptide-based vaccine that is protective in all age groups and immunogenic to all pneumococci serotypes globally. A conserved choline binding domain binds the choline binding protein (CbpA) to the phosphorylcholine moiety of the cell wall of Streptococcus pneumoniae (Gosink et al., 2000). Pneumococcal surface protein A (PspA) is a surface-exposed protein virulence agent for *S. pneumonia* (Tu et al., 1999). The two most significant protective surface antigens, PspA and CbpA, have been suggested as potential vaccine candidates (Miyaji *et al.,* 2015). Pneumococcal histidine triad protein D (PhtD) is a potential protein for a next-generation pneumococcal vaccine (Yun et al., 2015).

In this study, we utilized reverse vaccinology and immunoinformatics approaches to search and design new vaccine candidates against *streptococcus pneumoniae*. Due to recent developments in the next-generation sequencing of the pathogen and the accessibility of protein sequence databases internationally, these in-silico based methodologies have showed promise for vaccine development (Flower et al., 2008). In contrast, the traditional methods for developing vaccines are difficult and time-consuming and mainly rely on the expression and purification of a sufficient quantity of antigens from the in-vitro culture model.

2. Materials and methods

Protein Sequences

Amino acid sequences of Choline-binding protein A, Pneumococcal surface protein A and Pneumococcal histidine triad protein D of Streptococcus pneumonia were downloaded from protein database of NCBI (https://www.ncbi.nlm.nih.gov/). The Vaxign version 2.0 beta server

(Xiang and He 2009), a vaccine target prediction and analysis tool based on reverse vaccinology, was utilized to analyze envelope protein as a potential vaccine target for designing vaccine candidates.

Proteins Sequence Analysis

The antigenicity of the envelope protein sequence was determined using the VaxiJen v2.0 server (https://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (Doytchinova and Flower 2007). The Protparam software was used to predict the physicochemical properties of the envelope protein, such as the instability index, grand average of hydropathicity (GRAVY), theoretical pI, and molecular weight (Walker 2005; Wilkins et al. 1999). The protein's allergenicity was determined using AllerTOP v.2.0 (Dimitrov et al. 2014).

Prediction of Linear B-Cell Epitopes

ABCpred (Saha and Raghava 2006) and BepiPred 2.0 (Larsen et al. 2006) were used to predict linear B cell epitopes. Consensus predicted Linear B cell epitopes from both methods were chosen. Using the VaxiJen v2.0 server, the antigenicity of the epitopes was identifed. The server AllerTOP v.2.0 was used to compute the allergenicity of epitopes (Dimitrov et al. 2014). AllerTOPv.2.0 is a webbased allergenicity prediction tool that produces accurate fndings independent of alignment. Furthermore, using an SVM-based algorithm, ToxinPred was used with default parameters to predict the toxicity of epitopes (Gupta et al. 2013).

Homology Modeling of the Proteins

The homology model of envelope protein was built using the SWISS-MODEL server (Waterhouse et al. 2018). The Swiss-Model server's Structure Assessment page (https://swissmodel.expasy.org/assess) was used to validate the model's quality using Ramachandran plots. Another method used to validate the model structure was ProSA-web (Wiederstein and Sippl 2007). If the Z-score (total model quality score) falls outside of the expected range for native proteins, the structure is likely to be incorrect.

Prediction of Conformational B Cell Epitopes

SEPPA 3.0 (Zhou et al. 2019) and Ellipro (Ponomarenko et al. 2008) were utilized in predicting conformational B-cell epitopes based on the 3D structure of a protein. Conformational B-cell epitopes that were predicted by both methods were chosen as final.

3. Results

Vaccine Target

Using the Vaxign version 2.0 beta server, the choline binding protein A, pneumococcal surface protein A and pneumococcal histidine triad protein PhtD were explored as a potential vaccine target (Xiang and He, 2009). The protein is an adhesion if the probability of adhesion is greater than 0.51. The predicted protein bears no similarity to proteins found in humans.

Table 1: Vaxign results

Protein Name	Localization (Probability)	Adhesin Probability	Trans-membrane Helices	Similar Human Protein
Choline binding protein A	Periplasmic (Prob.=0.86)	0.713	0	-

Pneumococcal surface protein A	Periplasmic (Prob.=0.98)	0.836	1	-
Pneumococcal histidine triad protein PhtD	Extracellular (Prob.=0.95)	0.185	0	-

Target proteins sequence Aanalysis

The Choline-binding protein A, Pneumococcal surface protein A and Pneumococcal histidine triad protein D physicochemical parameters, such as the instability index, grand average of hydropathicity (GRAVY), theoretical pI, and molecular weight, were predicted using the Protparam software (Walker, 2005; Wilkins et al., 1999). The allergenicity of the protein was evaluated using AllerTOP v.2.0 (Dimitrov et al., 2014).

Table 2: Result of ProtParam tool

Protein Name	Instability index	GRAVY	Theoretical pI	Molecular weight (Da)	Antigenicity	allergenicity
Choline binding protein A	46.47	-1.149	5.73	77762.13	0.8312	Non-allergen
Pneumococcal surface protein A	40.52	-0.926	4.83	82764.23	0.6497	Non-allergen
Pneumococcal histidine triad protein PhtD	41.76	-0.863	5.22	95225.90	0.6323	Non-allergen

The protein antigenicity scores indicate an excellent vaccine antigenic feature. The proteins were non-allergenic. The proteins have a negative GRAVY value, indicating that the protein was hydrophilic and had a high interaction with water molecules.

Linear B-Cell Epitopes Analysis

Two types of bioinformatics tools searched for the full length sequence of envelope protein for probable sequential B-cell epitopes. The ABCpred server discovered a total of epitopes with a threshold of 0.80, as shown in tables 3-5. The BepiPred 2.0 tool predicted optimal B-cell epitopes at a threshold of 0.35, as shown in tables 6-8. Overlapped epitopes were presented as bold in tables 3-8. Antigenicity, toxicity and allergenicity of identifed B-cell epitopes were shown in tables 3-8. Epitopes with antigenic, non-toxic, and non-allergenic properties that were often predicted by the ABCpred and BepiPred 2.0 tools were chosen. On this basis, consensus linear B-cell epitopes, Choline binding protein A ($_{344-359}$KKKAEDQKEEDRRNYP, $_{281-296}$DAKEQGKPKGRAKRGV, $_{191-206}$EKKAKDQKEEDRRNYP, $_{314-329}$DSSVGEETLPSPSLKP), Pneumococcal surface protein A ($_{68-83}$AEDAQKKYEDDQKRTE, $_{41-56}$SSLEKKYEEAKAKADT) and Pneumococcal histidine triad protein D ($_{593-608}$TDHQDSGNTEAKGAEA, $_{587-602}$GLTPPSTDHQDSGNTE, $_{38-53}$RVSYIDGDQAGQKAEN) were identifed.

Table 3: Identifed B-cell epitopes on Choline binding protein A using ABCpred tool.

Sl. No.	Start position	ABCPRED predicted B cell epitope	ABCPRED Score	Antigenicity	Antigen/ non- antigen	Toxicity	Allergenicity

1	294	RGVPGELATPDKKEND	0.94	0.8972	Antigen	Non-Toxin	Non-allergen
2	521	NGMWYFYNTDGSMATG	0.92	0.5268	Antigen	Non-Toxin	Allergen
3	541	NGSWYYLNSNGAMATG	0.91	0.4910	Antigen	Non-Toxin	Non-allergen
4	344	**KKKAEDQKEEDRRNYP**	0.90	**1.6945**	Antigen	Non-Toxin	Non-allergen
5	153	SSSSDSSTKPEASDTA	0.90	1.6172	Antigen	Non-Toxin	Allergen
6	167	TAKPNKPTEPGEKVAE	0.89	0.6756	Antigen	Non-Toxin	Allergen
7	641	GDTWYYLEASGAMKAS	0.88	0.5430	Antigen	Non-Toxin	Allergen
8	601	NGSWYYLNANGSMATG	0.88	0.6817	Antigen	Non-Toxin	Allergen
9	581	NGSWYYLNANGSMATG	0.88	0.6817	Antigen	Non-Toxin	Allergen
10	561	NGSWYYLNANGSMATG	0.88	0.6817	Antigen	Non-Toxin	Allergen
11	281	**DAKEQGKPKGRAKRGV**	0.88	**1.1760**	Antigen	Non-Toxin	Non-allergen
12	191	**EKKAKDQKEEDRRNYP**	0.88	**1.7306**	Antigen	Non-Toxin	Non-allergen
13	672	GALAVNTTVDGYGVNA	0.87	1.3175	Antigen	Non-Toxin	Allergen
14	621	NGSWYYLNANGDMATG	0.87	0.7437	Antigen	Non-Toxin	Allergen
15	271	EAEEEAKRRADAKEQG	0.87	1.8400	Antigen	Non-Toxin	Allergen
16	44	TQVPTSSNRANESQAE	0.86	1.1458	Antigen	Non-Toxin	Non-allergen
17	314	**DSSVGEETLPSPSLKP**	0.86	**1.2286**	Antigen	Non-Toxin	Non-allergen
18	161	KPEASDTAKPNKPTEP	0.85	0.6686	Antigen	Non-Toxin	Allergen
19	650	SGAMKASQWFKVSDKW	0.84	0.5275	Antigen	Non-Toxin	Non-allergen
20	452	APKAEKPAPAPKPENP	0.84	0.4972	Antigen	Non-Toxin	Non-allergen
21	288	PKGRAKRGVPGELATP	0.84	0.6762	Antigen	Non-Toxin	Non-allergen
22	487	RRSEEEYNRLTQQQPP	0.83	0.8896	Antigen	Non-Toxin	Allergen
23	444	AEQPQPAPAPKAEKPA	0.83	0.5915	Antigen	Non-Toxin	Non-allergen
24	233	KVKANEPRDEQKIKQA	0.83	1.2241	Antigen	Non-Toxin	Non-allergen
25	207	TITYKTLELEIAESDV	0.83	1.0681	Antigen	Non-Toxin	Allergen
26	635	TGWVKDGDTWYYLEAS	0.82	-0.0193	Non-	Non-Toxin	Non-allergen
27	383	ELVKEEAKEPRNEEKV	0.82	0.6050	Antigen	Non-Toxin	Allergen
28	662	SDKWYYVNGSGALAVN	0.81	0.5964	Antigen	Non-Toxin	Non-allergen
29	58	AEQGEQPKKLDSERDK	0.81	1.3787	Antigen	Non-Toxin	Non-allergen
30	515	TGWKQENGMWYFYNTD	0.81	-0.2032	Non-	Non-Toxin	Non-allergen
31	424	KAEEEAKRKAAEEDKV	0.81	1.5535	Antigen	Non-Toxin	Allergen
32	367	ELEIAESDVEVKKAEL	0.81	1.5548	Antigen	Non-Toxin	Allergen
33	214	ELEIAESDVEVKKAEL	0.81	1.5548	Antigen	Non-Toxin	Allergen

Table 4: Identifed B-cell epitopes on Pneumococcal surface protein A using ABCpred tool.

Sl. No.	Start position	ABCPRED predicted B cell epitope	ABCPRED Score	Antigenicity	Antigen/ non-antigen	Toxicity	Allergenicity
1	509	EKPAEEPTQPEKPATP	0.96	-0.0355	Non- Antigen	Non-Toxin	Allergen
2	425	AELEKTQKELDAALNE	0.95	0.6800	Antigen	Non-Toxin	Allergen
3	68	**AEDAQKKYEDDQKRTE**	0.93	1.5308	Antigen	Non-Toxin	Non-allergen
4	653	NGSWYYLNANGAMATG	0.92	0.5753	Antigen	Non-Toxin	Allergen
5	552	NGSWYYLNANGAMATG	0.92	0.5753	Antigen	Non-Toxin	Allergen
6	101	LVVQNAYKEYREVQNQ	0.92	0.4934	Antigen	Non-Toxin	Non-allergen
7	41	**SSLEKKYEEAKAKADT**	0.91	1.2360	Antigen	Non-Toxin	Non-allergen
8	117	RSKYKSDAEYQKKLTE	0.91	0.7655	Antigen	Non-Toxin	Non-allergen
9	560	ANGAMATGWVKDGDTW	0.90	0.3381	Non- Antigen	Non-Toxin	Allergen
10	436	AALNELGPDGDEEETP	0.89	0.9689	Antigen	Non-Toxin	Non-allergen
11	693	GDTWYYLEASGAMKAS	0.88	0.5430	Antigen	Non-Toxin	Allergen
12	673	NGSWYYLNANGSMATG	0.88	0.6817	Antigen	Non-Toxin	Allergen
13	572	GDTWYYLEASGAMKAS	0.88	0.5430	Antigen	Non-Toxin	Allergen
14	532	NGMWYFYNTDGSMAIG	0.88	0.2420	Non- Antigen	Non-Toxin	Allergen
15	4	KKMILTSLASVAILGA	0.88	-0.2243	Non- Antigen	Non-Toxin	Non-allergen
16	681	ANGSMATGWVKDGDTW	0.87	0.4058	Antigen	Non-Toxin	Allergen
17	661	ANGAMATGWAKVNGSW	0.87	0.0555	Non- Antigen	Non-Toxin	Non-allergen
18	633	NGSWYYLNANGDMATG	0.87	0.7437	Antigen	Non-Toxin	Allergen
19	613	NGSWYYLNANGDMATG	0.87	0.7437	Antigen	Non-Toxin	Allergen
20	645	MATGWAKVNGSWYYLN	0.86	0.0213	Non- Antigen	Non-Toxin	Allergen
21	361	DPEGKTQDELDKEAEE	0.85	1.6062	Antigen	Non-Toxin	Non-allergen
22	280	DPEGKTQDELDKEAEE	0.85	1.6062	Antigen	Non-Toxin	Non-allergen
23	702	SGAMKASQWFKVSDKW	0.84	0.5275	Antigen	Non-Toxin	Non-allergen
24	581	SGAMKASQWFKVSDKW	0.84	0.5275	Antigen	Non-Toxin	Non-allergen
25	724	GALAVNTTVDGYKVNA	0.82	1.1036	Antigen	Non-Toxin	Allergen
26	687	TGWVKDGDTWYYLEAS	0.82	-0.0193	Non- Antigen	Non-Toxin	Non-allergen
27	593	SDKWYYVNSNGAMATG	0.82	0.6710	Antigen	Non-Toxin	Non-allergen
28	566	TGWVKDGDTWYYLEAS	0.82	-0.0193	Non- Antigen	Non-Toxin	Non-allergen
29	374	AEEAELDKKADELQNK	0.82	1.4427	Antigen	Non-Toxin	Allergen
30	293	AEEAELDKKADELQNK	0.82	1.4427	Antigen	Non-Toxin	Allergen
31	20	GFVTSQPTFVRAEESP	0.82	0.3514	Non- Antigen	Non-Toxin	Allergen
32	134	DSKIEKARKEQQDLQN	0.82	1.0510	Antigen	Non-Toxin	Non-allergen
33	526	TGWKQENGMWYFYNTD	0.81	-0.2032	Non- Antigen	Non-Toxin	Non-allergen
34	455	PQPEQPAPAPKPEQPA	0.81	0.4618	Antigen	Non-Toxin	Allergen
35	233	KLLAGADPDDGTEVIE	0.81	0.3124	Non- Antigen	Non-Toxin	Allergen
36	153	EVRAVVVPEPNALAET	0.81	0.4069	Antigen	Non-Toxin	Allergen

Table 5: Identifed B-cell epitopes on Pneumococcal histidine triad protein D using ABCpred tool.

Sl. No.	Start position	ABCPRED predicted B cell epitope	ABCPRED Score	Antigenicity	Antigen/ non-antigen	Toxicity	Allergenicity
1	718	EKPQTEKPEEDKEHDE	0.95	1.1188	Antigen	Non-Toxin	Non-allergen
2	543	DPRDITSDEGDAYVTP	0.95	0.8075	Antigen	Non-Toxin	Allergen
3	593	**TDHQDSGNTEAKGAEA**	0.93	2.2174	Antigen	Non-Toxin	Non-allergen
4	733	EVSEPTHPESDEKENH	0.92	0.7907	Antigen	Non-Toxin	Non-allergen
5	646	HYHNIKFEWFDEGLYE	0.92	0.5603	Antigen	Non-Toxin	Non-allergen
6	365	PSPQPAPNPQPAPSNP	0.92	0.7768	Antigen	Non-Toxin	Non-allergen
7	298	DPAQITSRTANGVAVP	0.92	0.8675	Antigen	Non-Toxin	Allergen
8	587	**GLTPPSTDHQDSGNTE**	0.91	1.1010	Antigen	Non-Toxin	Non-allergen
9	354	QPSPQSTPEPSPSPQP	0.91	0.9495	Antigen	Non-Toxin	Non-allergen
10	80	VTSHGDHYHYYNGKVP	0.89	0.4825	Antigen	Non-Toxin	Non-allergen
11	533	KYTTEDGYIFDPRDIT	0.89	0.3094	Non- antigen	Non-Toxin	Allergen
12	502	LAPIRHPERLGKPNAQ	0.89	-0.0680	Non- antigen	Non-Toxin	Non-allergen
13	679	VEHPNERPHSDNGFGN	0.88	0.5643	Antigen	Non-Toxin	Allergen
14	637	GSLIIPHYDHYHNIKF	0.88	0.0239	Non- antigen	Non-Toxin	Allergen
15	522	DDEIQVAKLAGKYTTE	0.88	0.6218	Antigen	Non-Toxin	Allergen
16	390	VRKVGDGYVFEENGVP	0.88	0.2753	Non- antigen	Non-Toxin	Allergen
17	131	DGKYYVYLKDAAHADN	0.88	0.2580	Non- antigen	Non-Toxin	Allergen
18	692	FGNASDHVQRNKNGQA	0.87	1.1123	Antigen	Non-Toxin	Allergen
19	334	ARIIPLRYRSNHWVPD	0.87	0.4248	Antigen	Non-Toxin	Non-allergen
20	233	KQGSRPSSSSSHNANP	0.87	1.2282	Antigen	Non-Toxin	Allergen
21	178	QGRYTTDDGYIFNASD	0.87	0.1254	Non- antigen	Non-Toxin	Non-allergen
22	786	HSVINAKIAEAEALLE	0.86	0.6246	Antigen	Non-Toxin	Non-allergen
23	657	EGLYEAPKGYSLEDLL	0.86	-0.6366	Non- antigen	Non-Toxin	Non-allergen
24	440	TDLPSSDREFYNKAYD	0.86	0.1436	Non- antigen	Non-Toxin	Non-allergen
25	38	**RVSYIDGDQAGQKAEN**	0.86	1.4136	Antigen	Non-Toxin	Non-allergen
26	221	SELAAAQAYWNGKQGS	0.86	0.6128	Antigen	Non-Toxin	Non-allergen
27	605	GAEAIYNRVKAAKKVP	0.85	0.2657	Non- antigen	Non-Toxin	Non-allergen
28	575	ERAAAQAYAKEKGLTP	0.85	0.3383	Non- antigen	Non-Toxin	Non-allergen
29	27	HQAGQVKKESNRVSYI	0.85	0.9079	Antigen	Non-Toxin	Non-allergen
30	87	YHYYNGKVPYDAIISE	0.84	0.3366	Non- antigen	Non-Toxin	Allergen
31	817	TGLKSSLLLGTKDNNT	0.84	0.7036	Antigen	Non-Toxin	Non-allergen
32	549	SDEGDAYVTPHMTHSH	0.84	0.9389	Antigen	Non-Toxin	Allergen
33	761	PSTDTEETEEEAEDTT	0.83	1.7995	Antigen	Non-Toxin	Non-allergen
34	473	DFEALDNLLERLKDVS	0.83	-0.1079	Non- antigen	Non-Toxin	Allergen
35	97	DAIISEELLMKDPNYQ	0.82	0.6227	Antigen	Non-Toxin	Non-allergen
36	345	HWVPDSRPEQPSPQST	0.82	0.4930	Antigen	Non-Toxin	Non-allergen
37	258	NLTVTPTYHQNQGENI	0.82	0.9029	Antigen	Non-Toxin	Non-allergen
38	246	ANPAQPRLSENHNLTV	0.82	0.8254	Antigen	Non-Toxin	Allergen

39	190	NASDIIEDTGDAYIVP	0.82	0.0396	Non- antigen	Non-Toxin	Allergen
40	796	AEALLEKVTDSSIRQN	0.81	0.7040	Antigen	Non-Toxin	Non-allergen
41	67	AEQIVIKITDQGYVTS	0.81	0.2565	Non- antigen	Non-Toxin	Non-allergen
42	424	KLAKQESLSHKLGAKK	0.81	1.0587	Antigen	Non-Toxin	Allergen
43	269	QGENISSLLRELYAKP	0.81	0.2912	Non- antigen	Non-Toxin	Allergen

Table 6: Identifed B-cell epitopes on Choline binding protein A using Bepipred tool.

Sl. No.	Start	End	Bepipred predicted B cell epitope	Antigenicity	Antigen/ Non-antigen	Toxicity	Allergenicity
1	36	79	HATENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVE	1.3498	Antigen	Non-Toxin	Allergen
2	89	97	SYAKSTKKR	1.1318	Antigen	Non-Toxin	Non-allergen
3	121	126	ESTSES	2.1131	Antigen	Non-Toxin	Allergen
4	143	208	VSKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEA**EKKAKDQKEEDRRNYP**TI	1.1727	Antigen	-	Non-allergen
5	236	260	ANEPRDEQKIKQAEAEVESKQAEAT	1.4259	Antigen	Non-Toxin	Allergen
6	266	363	KTDREEAEEEAKRRA**DAKEQGKPKGRAKRGV**PGELATPDKKENDAKSS**DSSVGEETLPSPSLKP**EKKVAEAEKKVEEA**KKKAEDQKEEDRRNYP**TNTY	1.3646	Antigen		Non-allergen
7	387	413	EEAKEPRNEEKVKQAKAEVESKKAEAT	1.4484	Antigen	Non-Toxin	Non-allergen
8	419	520	KTDRKKAEEEAKRKAAEEDKVKEKPAEQPQPAPAPKAEKPAPAPKPENPAEQPKAEK	0.9230	Antigen		Non-allergen
			PADQQAEEDYARRSEEEYNRLTQQQPPKTEKPAQPSTPKTGWKQE				
9	631	644	GDMATGWVKDGDTW	0.1421	Non-antigen	Non-Toxin	Allergen

Table 7: Identifed B-cell epitopes on Pneumococcal surface protein A using Bepipred tool.

Sl. No.	Start	End	Bepipred predicted B cell epitope	Antigenicity	Antigen/ Non-Antigen	Toxicity	Allergenicity
1	26	95	PTFVRAEESPQVVEK**SSLEKKYEEAKAKADT**AKKDYETAKKK**AEDAQKKYEDDQKRTE**EKARKEAEASQK	1.2862	Antigen		Non-allergen
2	108	132	KEYREVQNQRSKYKSDAEYQKKLTE	0.9339	Antigen	Non-Toxin	Non-allergen
3	135	149	SKIEKARKEQQDLQN	0.6579	Antigen	Non-Toxin	Non-allergen
4	159	184	VPEPNALAETKKKAEEAKAEEKVAKR	1.1316	Antigen	Non-Toxin	allergen
5	219	227	QEVATAQHQ	0.7589	Antigen	Non-Toxin	Non-allergen
6	237	246	GADPDDGTEV	1.2765	Antigen	Non-Toxin	allergen
7	252	260	KKGEAELNA	1.4978	Antigen	Non-Toxin	Non-allergen
8	263	268	AELAKK	0.6035	Antigen	Non-Toxin	allergen
9	279	307	LDPEGKTQDELDKEAEEAELDKKADELQN	1.3580	Antigen	Non-Toxin	allergen
10	325	334	GADPEDDTAA	0.7679	Antigen	Non-Toxin	allergen
11	360	388	LDPEGKTQDELDKEAEEAELDKKADELQN	1.3580	Antigen	Non-Toxin	allergen
12	407	415	ADSEDDTAA	1.4073	Antigen	Non-Toxin	allergen
13	425	434	AELEKTQKEL	0.4598	Antigen	Non-Toxin	allergen
14	439	531	NELGPDGDEEETPAPAPQPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPATPKTGWKQE	0.5338	Antigen		allergen
15	567	575	GWVKDGDTW	0.2188	Non-antigen	Non-Toxin	allergen
16	643	652	GDMATGWAKV	0.0081	Non-antigen	Non-Toxin	Non-allergen
17	683	696	GSMATGWVKDGDTW	0.1555	Non-antigen	Non-Toxin	allergen

Table 8: Identifed B-cell epitopes on Pneumococcal histidine triad protein D using Bepipred tool.

Sl. No.	Start	End	Bepipred predicted B cell epitope	Antigenicity	Antigen/ Non-Antigen	Toxicity	Allergenicity
1	27	37	HQAGQVKKESN	1.2930	Antigen	Non-Toxin	allergen
2	39	66	VSYIDGDQAGQKAENLTPDEVSKREGIN	1.0822	Antigen	Non-Toxin	Non-allergen

3	77	89	QGYVTSHGDHYHY	0.1132	Non- Antigen	Non-Toxin	Non-allergen
4	142	150	AHADNIRTK	1.9058	Antigen	Non-Toxin	allergen
5	152	188	EIKRQKQERSHNHNSRADNAVAAARA QGRYTTDDGYI	0.9884	Antigen	Non-Toxin	Non-allergen
6	194	204	IIEDTGDAYIV	0.0874	Non- Antigen	Non-Toxin	allergen
7	228	254	AYWNGKQGSRPSSSSSHNANPAQPRL S	1.0020	Antigen	Non-Toxin	allergen
8	258	272	NLTVTPTYHQNQGEN	1.1491	Antigen	Non-Toxin	Non-allergen
9	285	291	LSERHVE	1.2704	Antigen	Non-Toxin	allergen
10	301	315	QITSRTANGVAVPHG	0.6120	Antigen	Non-Toxin	allergen
11	345	383	HWVPDSRPEQPSPQSTPEPSPSPQPAPN PQPAPSNPIDE	0.6165	Antigen	Non-Toxin	Non-allergen
12	397	421	YVFEENGVPRYIPAKDLSAETAAGI	0.7108	Antigen	Non-Toxin	Non-allergen
13	436	449	GAKKTDLPSSDREF	0.1441	Non-antigen	Non-Toxin	allergen
14	506	525	RHPERLGKPNAQITYTDDEI	0.4198	Antigen	Non-Toxin	allergen
15	533	542	KYTTEDGYIF	0.3892	Non-antigen	Non-Toxin	Non-allergen
16	545	557	RDITSDEGDAYVT	0.8039	Antigen	Non-Toxin	Non-allergen
17	570	608	SLSEAERAAAQAYAKEK**GLTPPSTDH QDSGNTEAKGAEA**	0.9017	Antigen	Non-Toxin	Non-allergen
18	659	667	LYEAPKGYS	-0.5959	Non-antigen	Non-Toxin	Non-allergen
19	680	784	EHPNERPHSDNGFGNASDHVQRNKNG QADTNQTEKPNEEKPQTEKPEEDKEH DEVSEPTHPESDEKENHVGLNPSADNL YKPSTDTEETEEEAEDTTDEAEIPQV	1.0322	Antigen	-	allergen
20	805	810	DSSIRQ	2.0682	Antigen	Non-Toxin	Non-allergen
21	828	835	KDNNTISA	1.3995	Antigen	Non-Toxin	allergen

Homology modeling of Choline binding protein A and tertiary structure validation

Homology modeling of the target protein's 3D structure was done in stages, commencing with a template structure search on the Swiss-Model server. From a large number of hits, a template structure of AlphaFold DB model of Q9KK36_STREE (UniProt Id: Q9KK36.1.A) was picked as the model's construction. The target sequence has 100% query coverage, and the sequence identity with the template sequence is 96.09%. Based on the template and target alignment, the Swiss-Model server built a homology model of the target sequence. The Swiss model/Structure assessment page was utilized to validate the tertiary structure of the model using Ramachandran plot analysis. Figure 1a shows a Ramachandran plot of the predicted model with 89.15% of residues in the favored region and 5.35% in the outlier region. The ProSA-web server calculates the total quality score for a given input structure and displays it in the context of all known

protein structures. Using the ProSA web server, the model protein Z score was -7.8, as shown in Fig. 1b, in broad black dot.

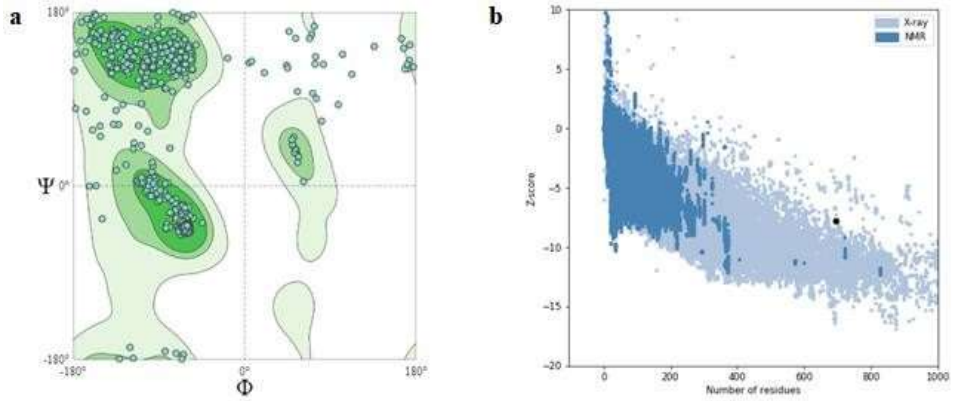

Fig. 1: The Ramachandran plot and the ProSA-web server were used to validate the tertiary structure of the model protein. a Ramachandran plot shows that the number of amino acid residues in the favorable region is 89.15%. b The ProSA-web result gives a Z score of -7.8

Homology modeling of Pneumococcal surface protein A and tertiary structure validation

From a large number of hits, a template structure of AlphaFold DB model of A0A4L8MNG6_STREE (UniProt Id: A0A4L8MNG6) was picked as the model's construction. The target sequence has 99% query coverage, and the sequence identity with the template sequence is 84.05%. Based on the template and target alignment, the Swiss-Model server built a homology model of the target sequence.

The Swiss model/Structure assessment page was utilized to validate the tertiary structure of the model using Ramachandran plot analysis. Figure 2a shows a Ramachandran plot of the predicted model with 89.76% of residues in the favored region and 5.12% in the outlier region. The ProSA-web server calculates the total quality score for a given input structure and displays it in the context of all known protein structures. Using the ProSA web server, the model protein Z score was -8.76, as shown in Fig. 2b, in broad black dot.

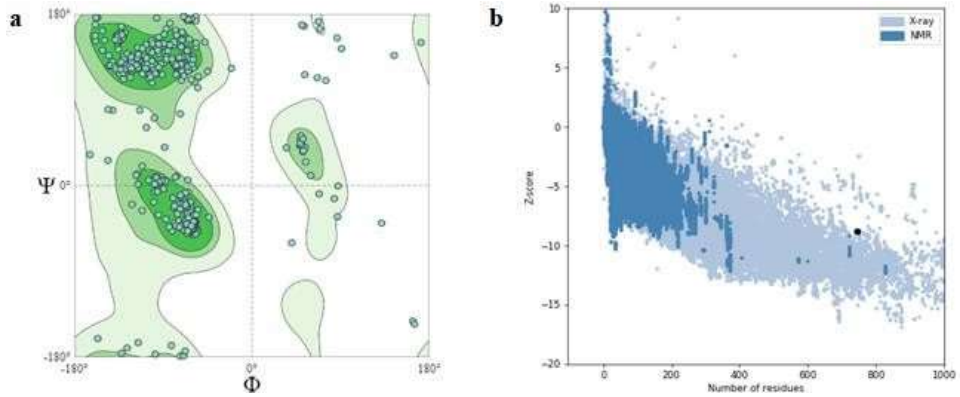

Fig. 2: The Ramachandran plot and the ProSA-web server were used to validate the tertiary structure of the model protein. a Ramachandran plot shows that the number of amino acid residues in the favorable region is 89.76%. b The ProSA-web result gives a Z score of -8.76

Homology modeling of Pneumococcal histidine triad protein D and tertiary structure validation

From a large number of hits, a template structure of AlphaFold DB model of Q8DQ08_STRR6 (UniProt Id: Q8DQ08) was picked as the model's construction. The target sequence has 100% query coverage, and the sequence identity with the template sequence is 100%. Based on the template and target alignment, the Swiss-Model server built a homology model of the target sequence.

The Swiss model/Structure assessment page was utilized to validate the tertiary structure of the model using Ramachandran plot analysis. Figure 3a shows a Ramachandran plot of the predicted model with 88.60% of residues in the favored region and 4.11% in the outlier region. The ProSA-web server calculates the total quality score for a given input structure and displays it in the context of all known protein structures. Using the ProSA web server, the model protein Z score was -12.44, as shown in Fig. 3b, in broad black dot.

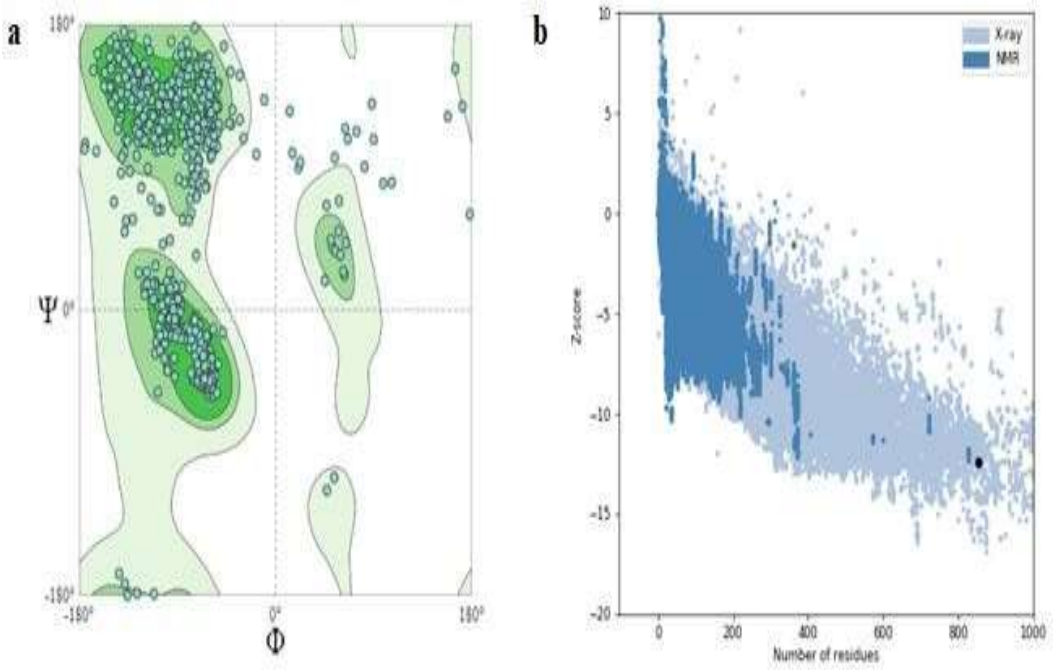

Fig. 3: The Ramachandran plot and the ProSA-web server were used to validate the tertiary structure of the model protein. a Ramachandran plot shows that the number of amino acid residues in the favorable region is 88.60%. b The ProSA-web result gives a Z score of -12.44

Conformational B-Cell Epitopes Analysis

Conformational B-cell epitopes on envelope protein were identifed by SEPPA 3.0 (Zhou et al. 2019) at a threshold of 0.17. 3D representations representation of the predicted discontinuous residues on proteins by SEPPA 3.0 were shown in fig. 5a, 6a &7a and predicted residues in fig. 5b, 6b &7b.

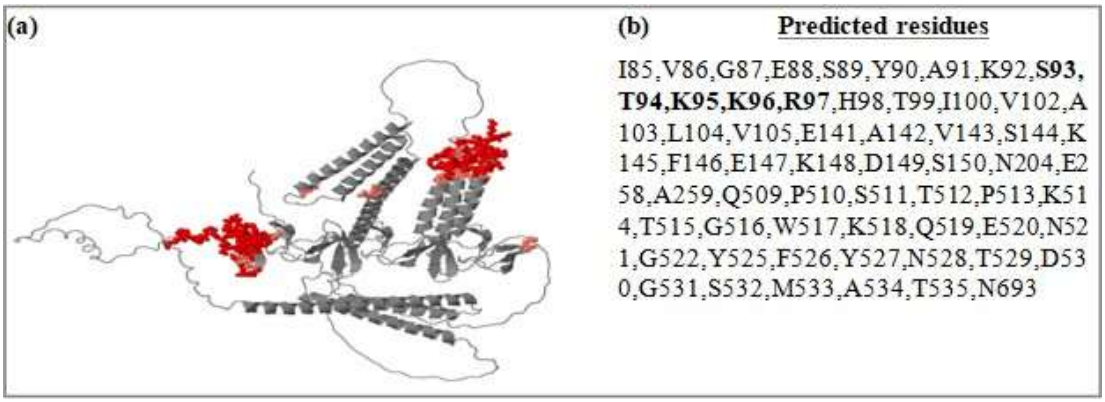

Fig. 4: Identifed conformational B-cell epitopes by SEPPA 3.0. a 3D representation of the predicted discontinuous residues on envelope protein was shown as balls. Color of balls was red when score>=0.2 and salmon when score>=0.17. b Predicted residues.

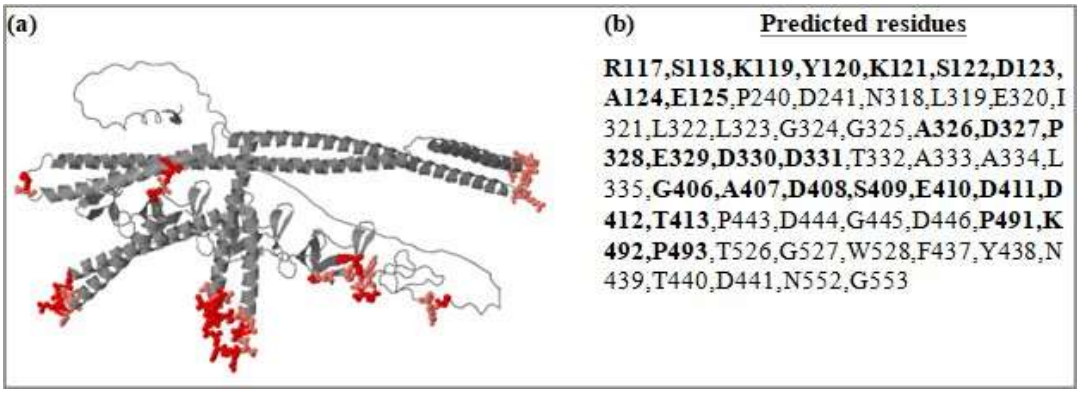

Fig. 5: Identifed conformational B-cell epitopes by SEPPA 3.0. a 3D representation of the predicted discontinuous residues on envelope protein was shown as balls. Color of balls was red when score>=0.2 and salmon when score>=0.17. b Predicted residues.

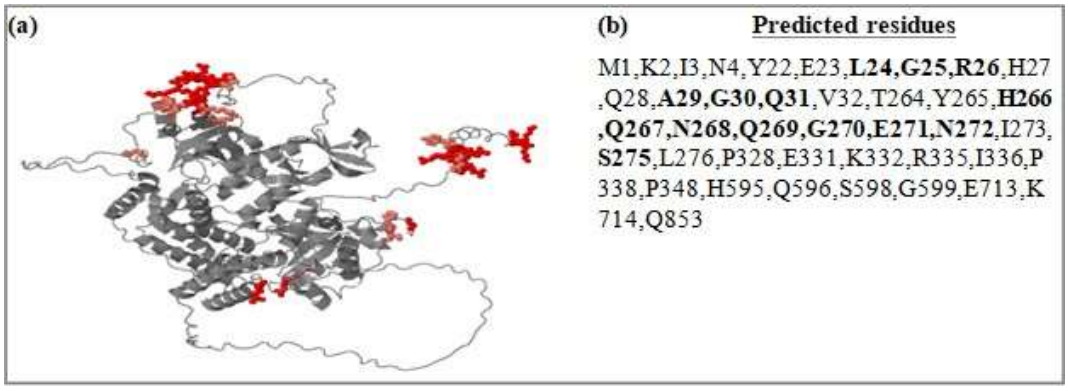

Fig. 6: Identifed conformational B-cell epitopes by SEPPA 3.0. a 3D representation of the predicted discontinuous residues on envelope protein was shown as balls. Color of balls was red when score>=0.2 and salmon when score>=0.17. b Predicted residues.

Ellipro (Ponomarenko et al. 2008) at a threshold of 0.8 predicted conformational B-cell epitopes, were shown in tables 9-11. B-cell epitope scanning and analysis using both SEPPA 3.0 and Ellipro tools predicted potent conformational B-cell epitopes, Choline binding protein A ($_{93-97}$STKKR), Pneumococcal surface protein A ($_{117-125}$RSKYKSDAE, $_{491-493}$PKP, $_{326-331}$ADPEDD, $_{406-413}$GADSEDDT) and Pneumococcal histidine triad protein D ($_{24-26}$LGR, $_{29-31}$AGQ, $_{266-272}$HQNQGEN) as a component of B-cell epitopes.

Table 9: Predicted Discontinuous Epitopes of Choline binding protein A

No.	Residues	Number of residues	Score
1	Q470, P471, K472, A473, E474, K475, P476, A477, D478, Q479	10	0.972
2	P458, A459, P460, A461, P462, K463, P464, E465, N466, P467, A468, E469	12	0.972
3	L496, T497, Q498, Q499	4	0.938
4	G295, V296, P297, G298, E299, L300, A301, T302, P303, D304, K305, K306, E307, N308, D309, A310, K311, S312, S313, D314, S315, S316, V317, G318, E319	25	0.912
5	S152, S153, S154, S155, S156, D157, S158, S159, T160, K161, P162, E163, A164, S165, D166, T167, A168, K169, P170, N171	20	0.888
6	Q500, P501, P502, K503, T504, E505	6	0.867
7	K442, P443, A444, E445, Q446, P447, Q448, P449, A450, P451, A452, P453, K454, A455, E456, K457	16	0.867
8	E320, T321, L322, P323, S324, P325, S326	7	0.85
9	G290, R291, A292, K293	4	0.835
10	K172, P173, T174, E175	4	0.817
11	**S93, T94, K95, K96, R97**	5	0.817
12	K506, P507, A508	3	0.811
13	M31, G32, S33, V34, V35, H36, A37, T38, E39, N40	10	0.804

Table 10: Predicted Discontinuous Epitopes of Pneumococcal surface protein A

No.	Residues	Number of residues	Score
1	E447, E448, E449, T450, P451, A452, P453, A454, P455, Q456, P457, E458, Q459, P460, A461, P462, A463, P464, K465, P466, E467, Q468, P469, A470, P471, A472, P473, K474, P475, E476, Q477, P478, A479, P480, A481, P482, K483, P484, E485	39	0.926
2	D98, V99, A100, L101, V102, V103, Q104, N105, A106, Y107, K108, E109, Y110, R111, E112, V113, Q114, N115, Q116, **R117, S118, K119, Y120, K121, S122, D123, A124, E125**, Y126, Q127, K128, K129, L130, T131, E132, V133, D134, S135, K136, I137, E138, K139, A140, R141, K142, E143, Q144, Q145, D146, L147, Q148, N149, K150, E153	54	0.919
3	A19, G20, F21, V22, T23, S24, Q25, P26, T27, F28, V29	11	0.862
4	Q486, P487, A488, P489, A490**, P491, K492, P493**	8	0.861

5	S714, D715, K716, L726, A727, V728, N729, T730, T731, V732, D733, G734, Y735, K736, V737, N738, A739, N740,G741, E742, W743, V744	22	0.847
6	R30, A31, E32, E33	4	0.827
7	**A326, D327, P328, E329, D330, D331**	6	0.821
8	**G406, A407, D408, S409, E410, D411, D412, T413**, A414, A415, N418	11	0.814

Table 11: Predicted Discontinuous Epitopes of Pneumococcal histidine triad protein D

No.	Residues	Number of residues	Score
1	L8, A9, G10, S11, V12, A13, V14, L15, A16, L17, S18, V19, C20, S21	14	0.975
2	H731, D732, E733, V734, S735, E736, P737, T738, H739, P740, E741, S742, D743, E744	14	0.963
3	E746, N747, H748, V749, G750, L751	6	0.958
4	K724, P725, E726, E727, D728, K729	6	0.93
5	N752, P753, S754, A755, D756, N757, L758, K760, P761, S762, T763, D764, T765, E766, E767, T768, E769	17	0.918
6	P238, S239, S240, S241, S242, S243, H244, N245, A246, N247, P248, A249, Q250, P251, R252	15	0.895
7	**L24, G25, R26**	3	0.89
8	E771, A772, E773	3	0.883
9	Q354, P355, S356, P357, Q358, S359, T360, P361, E362, P363, S364, P365, S366, P367, Q368, P369, A370, P371, N372, P373	20	0.881
10	**A29, G30, Q31**	3	0.872
11	K719, P720, Q721	3	0.86
12	L253, S254, E255, N256, H257	5	0.857
13	D774, T775, T776, D777, E778, A779, E780, I781, P782, Q783	10	0.83
14	P263, T264, **H266, Q267, N268, Q269, G270, E271**	8	0.829
15	K34, E35, S36, N37	4	0.819
16	P849, T850, P851, I852	4	0.817
17	**N272**, S274, S275, R278	4	0.816

Conclusion

Epitope search enjoys an extra benefit to additional limited down the antigen evaluating for extremely short unambiguous regions, subsequently giving a chance where protein based control can be utilized to cooperative energies and select the proper resistant reaction type. This research identifes new potential B-cell epitopes for fighting Streptococcus pneumonia infections and may lead to the development of Streptococcus pneumonia vaccines. Because the fndings of this study are based on computational methodologies, laboratory experiments in-vitro and in-vivo are required to validate this work.

Author Contributions

All authors reviewed the manuscript

Declarations

Conflict of interest

The author declares that there is no conflict of interest.

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