ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2025;11(1):1-12 10.52547/iem.11.1.1

Comprehensive Analysis of Four Major Surface Proteins for Vaccine Design against *Klebsiella pneumoniae*

ARTICLE INFO

Article Type
Original Article

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How to cite this article
Vaez H., Vaez V. Comprehensive Analysis of Four Major Surface Proteins for Vaccine Design against Klebsiella pneumoniae. Infection Epidemiology and Microbiology. 2025;11(1): 1-12.

Article History
Received: April 19, 2024
Accepted: December 21, 2024
Published: February 22, 2025

ABSTRACT

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is responsible for life-threatening infections, given that it is usually resistant to antibacterial drugs. Due to the restricted antibiotic options for the treatment of resistant *K. pneumoniae* infections and the critical role of humoral immune responses in preventing infectious diseases, the present in silico study aimed to investigate fimbriae (type 1 and type 3), outer membrane protein A (OmpA), and outer membrane protein K35 (OmpK35) to find appropriate epitopes for vaccine development.

Materials & Methods: Several independent bioinformatics servers including IEDB, ABCpred, VaxiJen, and EMBOSS were applied to identify appropriate linear epitopes (B-cell and T-cell). Conformational epitopes were also predicted using Ellipro and Discotope programs. The Antigenic Peptide Prediction server was used to confirm the identified epitopes. Molecular characteristics, toxicity, human similarity, and allergenicity were investigated.

Findings: The results demonstrated that the investigated proteins were highly immunogenic. In the first step, 25 epitopes were identified in the investigated proteins. After applying different exclusion criteria, the final epitope of each investigated protein was selected. The final epitopes of fimbriae (type 1 and type 3), OmpK35 and OmpA were located in 28-49, 26-53, 271-291, and 288-299 regions, respectively. Allergenicity, toxicity, and human similarity were negative for the predicted epitopes.

Conclusion: The present study results introduced four reliable B-cell and T-cell epitopes (each for one investigated protein) with appropriate physicochemical characteristics. The proposed epitopes could be used in vaccine development against *K. pneumoniae* after further in vitro and in vivo studies.

Keywords: *Klebsiella pneumoniae,* OmpK35, fimbriae, Outer membrane protein

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is responsible for different types of infections with considerable mortality and morbidity in humans, including pneumonia, liver abscess, urinary tract infections, bloodstream infections, meningitis, and wound infections. Treatment of *K. pneumoniae* infections has become more difficult owing to multiple antibiotic resistance mechanisms in this bacterium and restricted access to alternative treatments [1-3].

K. pneumoniae strains are categorized into two pathotypes, namely hypervirulent *K. pneumoniae* (hvKP) and classical *K. pneumoniae* (cKP). Importantly, hvKp is an evolving pathotype recognized to be more virulent than cKp. The hvKP pathotype has been demonstrated to have the ability to acquire multiple mobile genetic elements, which confer resistance to various antibiotics and cause the spread of invasive infections with high mortality [4].

K. pneumoniae vaccine could play a pivotal role in preventing and reducing the prevalence of *K. pneumoniae* infections among patients, especially those who are at higher risk of developing severe illnesses, including immunocompromised individuals and hospitalized patients [5].

Several vaccine strategies have been tested for *K. pneumoniae*, including inactivated whole-cell vaccines, live attenuated vaccines (genetically modified bacteria), recombinant vaccines, and capsular-polysaccharide-based vaccines; however, no approved vaccine is available for *K. pneumoniae* infections [5, 6].

Several independent studies have revealed that recombinant outer membrane protein (OMP)-based vaccines are promising vaccine candidates (especially in combination with other proteins) against *K. pneumoniae* infections; however, the cross-reactivity with other molecules expressed by the host microbiota is known as a serious challenge that should be resolved [7]. Bioinformatics tools are cost-effective and reliable methods for selecting specific immunogenic epitopes that do not show cross-reactivity [8, 9].

Two main types of fimbriae (type 1 and type 3) have been reported in *K. pneumoniae.* These proteins are related to adhesion to host cells (epithelium and mucous membranes) and biofilm formation, playing a significant role in pathogenicity [10]. Studies conducted on immunization against *K. pneumoniae* have indicated that purified fimbriae proteins could stimulate the immune system and induce the production of chemokines [9-11]. *K. pneumoniae* OMPs were found to be important for pathogenesis, transport of molecules, and membrane integrity. Outer membrane protein A (OmpA) and outer membrane protein K35 (OmpK35) contribute to adhesion to eukaryotic cells, immune resistance, and antibiotic resistance [11].

An epitope is defined as a part of an antigen, which is recognized by the host's immune system, specifically by the corresponding antigen receptor on the immune cell, and elicits immune responses to the invading pathogen [12]. The advantage of epitope-based vaccines is that they could decrease biological risks associated with other types of immunization and provide maximal therapeutic efficacy with minimal side effects and cross-reactivity [13].

Objectives: Given the substantial burden of *K. pneumoniae* infections on public health and due to the critical role of humoral immune responses in preventing *K. pneumoniae* infections, the present in silico study aimed to investigate type 1 and 3 fimbriae as well as OmpA and OmpK35 to find appropriate epitopes (B-cell and T-cell) for vaccine development against *K. pneumoniae.*

Materials & Methods

Protein characterization: The full sequenc-

es of fimbriae (type 1 and type 3) proteins (accession number: WP240245605.1 and WP346724182.1), as well as OmpK35 and OmpA (accession number: WP318526724.1 and WP217048700.1), were obtained from https://www.ncbi.nlm.nih.gov/protein.

In the first step, the sequence of the retrieved protein was compared to sequence databases using the Blastp server (https://blast.ncbi.nlm.nih.gov/Blast). Then the biological function and three-dimensional (3D) structures of the retrieved proteins were investigated by GOR IV (https://npsa-prabi.ibcp.fr) [14] and Protparam (https://web.expasy.org/protparam) [15]. The antigenicity of the retrieved proteins was also evaluated by the VaxiJen server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [16]. The instructions provided by the servers were applied.

Linear B-cell epitope prediction: Epitope prediction was performed by the IEDB server (http://tools.iedb.org/main/bcell/) [17]. In this server, epitope prediction is performed based on different tools, including beta-turn [18], flexibility [19], surface accessibility [20], antigenicity [21], and hydrophilicity [22]. All sequences were submitted to the server and analyzed based on the tools recommendations. The outputs of each tool (chart and table) were evaluated independently, and regions predicted as epitopes by at least four out of five applied tools were subjected to further analysis. To achieve the best prediction, Bepipred Linear Epitope Prediction 2.0 (http://tools.iedb.org/main/bcell/) [23], Bepipred Linear Epitope Prediction (http://tools.iedb.org/main/bcell/) [24], ABCpred (https://webs.iiitd.edu.in/raghava/abcpred/ABC) [25], Antigenic Peptide Prediction server (http://imed.med.ucm.es/Tools/index.html), and EMBOSS server were also applied (https://www.bioinformatics.nl/cgi-bin/emboss/antigenic).

Discontinuous B-cell epitope prediction: Conformational B-cell epitope prediction is based on protein tertiary structure (3D). Therefore, the sequence of the proteins was submitted to the SWISS-MODEL server (https://swissmodel.expasy.org/) [26], and analyzed based on server recommendations. In this server, the 3D structure of a protein is built in four steps, including i) structural template(s) identification, ii) target sequence alignment, iii) model-building, and iv) model quality assessment. The quality and accuracy of the created models were investigated using GMQE (Global Model Quality Estimate) and QMEANDisCo score (higher numbers indicating higher expected quality). In addition, the quality of the created models was evaluated by generating Ramachandran plots using the RAMPAGE server (https://mordred.bioc.cam.ac.uk/). The Ramachandran plot, by plotting the phi (ϕ) and psi (ψ) dihedral angles, assesses the sterically allowed and disallowed conformations of the protein backbone, determining which torsional angles are permitted and could provide insights into the structure of peptides [27]. After complimenting the 3D structure of the protein, the PDB file was downloaded from the SWISS-MODEL server. Finally, two online bioinformatic servers, including Discotope [28] and Ellipro (http://tools.iedb.org/ellipro/) [29], were applied to assess conformational B-cell epitopes. The downloaded PDB file was submitted (using default parameters) to these two indicated servers.

Toxicity, human similarity, and allergenicity: The predicted epitopes were submitted to Toxinpred (https://webs.iiitd.edu.in/raghava/toxinpred) [30], Protein BLAST server at NCBI (https://blast.ncbi.nlm.nih.gov/Blast), and AllercatPro (https://allercatpro.bii.a-star.edu.sg/) [31] for evaluation of toxicity, similarity to human protein, and allergenicity, respectively. The predicted epitopes with more than 90% similarity in identity

and coverage values with human peptides were excluded.

Molecular characteristics of final epitopes: The molecular characteristics of the final epitopes, such as instability index, half-time, aliphatic index, isoelectric pH (pI), and 3D structure of peptides, were evaluated by Expasy (https://web.expasy.org/protparam/) [32] and PEP-FOLD4 servers (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD4/) [33].

T cell (CD8+ and CD4+) epitope (Class I and II major histocompatibility complex (MHC)) prediction: The ability of epitopes to stimulate T cells is crucial and depends on their ability to bind to human leukocyte antigen (HLA) alleles. Therefore, the final epitopes were checked for their ability to bind to HLA-I and HLA-II. The final epitope of each protein was checked using two independent MHC class I and II prediction tools to evaluate their ability to bind to HLA alleles. The used servers were IEDB (http://tools.iedb.org) and SYFPEITHI (http://www.syfpeithi.de). In the IEDB server, cutoff ≤1 and ≤10 percentile ranks were used for MHC class I and II, respectively. In SYFPEITHI, cutoff ≥ 10 scores were used for both MHC class I and II. Eleven highly frequent HLA-I and HLA-II alleles were obtained from the Allele Frequency Net Database (http://www.allelefrequencies.net/), namely HLA 01:01, HLA 02:01, HLA 03:01, HLA 11:01, HLA 24:02, HLB 08:01, HLB 35:01, HLB 51:01, HLA-DRB1 03:01, HLA-DRB1 07:01, and HLA-DRB1 15:01.

Findings

Protein characterization: The complete sequences of type 1 and type 3 fimbriae and OmpA and OmpK35 proteins contained 182, 331, 356, and 359 amino acids, respectively. Blastp analysis results revealed that these proteins were conserved among *Klebsiella spp*., with more than 98% identity for OmpA, OmpK35, and type 3 fimbria as well as more than 86% identity for type 1 fimbria (data not shown). Based on the Protparam server, the molecular weight (MW) and isoelectric point were determined to be 18093.11 Da and 4.61 for type 1 fimbria, 35093.87 Da and 9.28 for type 3 fimbria, 38026.58 Da and 6.84 for OmpA, and 39540.26 Da and 4.66 for OmpK35, respectively. In addition, the instability index and aliphatic index were estimated to be 12.98 and 87.58 for type 1 fimbria, 32.58 and 81.87 for type 3 fimbria, 25.08 and 71.85 for OmpA, and 20.84 and 60.97 for OmpK35, respectively, indicating that the proteins have a long half-life (30 hours in mammalian reticulocyte, >20 hours in yeast, and >10 hours in *Escherichia coli*). The secondary structure of the proteins was mainly as follows: random coil 42.86%, alpha helix 32.97%, and extended strand 24.18% for type 1 fimbria; random coil 54.08%, extended strand 36.86%, and alpha helix 9.06% for type 3 fimbria; random coil 53.93%, alpha helix 27.81%, and extended strand 18.26% for OmpA; and random coil 47.35%, extended strand 27.58%, and alpha helix 25.07% for OmpK35. Based on the prediction of the VaxiJen server (cutoff 0.4), type 1 fimbria, type 3 fimbria, OmpA, and OmpK35 were identified to be antigenic with antigenicity scores of 0.8159, 0.8196, 0.6786, and 0.7654, respectively.

Linear B-cell epitope prediction: Linear B cell epitope prediction was carried out in the first step using IEDB and ABCpred. The charts and tables provided by each tool on the IEDB server were investigated separately. Epitopes were selected based on the scores reported for each region (scores higher than the threshold). In total, 25 epitopes were selected: five in type 1 fimbria, eight in type 3 fimbria, seven in OmpA, and five in OmpK35. Epitopes predicted by at least four of the five tools were subjected to further confirmation using ABCpred (Table 1). In Table 2, the

results of the antigenicity evaluation by the EMBOSS sever are presented, the region with a higher score shows a higher potency for antigenicity.

Discontinuous B-cell epitope prediction: The 3D structures of type 1 and 3 fimbriae, OmpA, and OmpK35 were built using the SWISS-MODEL server based on the provided instructions. The proposed models were built successfully since they had high QMEANDisCo scores. The QMEANDisCo scores for type 1 and 3 fimbriae, OmpA, and OmpK35 were as follows: 0.80±0.07, 0.54±0.05, 0.65±0.06, and 0.94±0.05, respectively. In addition, Ramachandran plots confirmed the quality of the models and showed the majority of

Table 1) Prediction of B-cell epitope (linear) by IEDB and ABCpred servers

Prediction Tool Epitope	Antigenicity	Beta turn	Flexibility	Hydrophilicity	Surface Accessibility	Bepipred1	Bepipred2	ABC pred
Type 1fimbria								
112QNSAAGS	-	+	+	+	+	-	+	+
170TANADAT	-	+	+	+	+	+	-	+
52DQTVQLG	+	+	+	+	+	-	-	+
28VNGGTVH	+	+	+	+	+	-	-	+
145 TTLNDGT	-	+	+	+	+	-	+	+
Type 3 fimbria								
27RLSSPTV	+	+	+	+	+	-	-	+
69 YRCTSGT	-	+	+	+	+	+	-	+
204 RRTDLKG	-	+	+	+	+	-	+	+
258 LNEKAGS	-	+	+	+	+	-	+	+
231 SETGYAN	-	+	+	+	+	+	-	+
64 PGGASYR	-	+	+	+	+	+	-	+
118 PDVFSSR	+	+	+	+	+	-	+	+
273 QVLKDGS	+	+	+	+	+	-	-	+
OmpA								
213 APAPAPE	+	+	+	+	+	+	+	+
293 DYPVAKG	+	+	-	+	+	+	-	+
278 AYNQQLS	+	+	+	+	+	+	+	+
345 KGYKEVV	+	-	+	+	+	-	+	+
23 PKDNTRY	-	+	+	+	+	+	-	+
20 QAAPKDN	-	+	+	+	+	+	-	+
88 AYKGSVD	+	+	+	+	-	-	+	+
OmpK35								
285 GYVQTKG	+	+	+	+	-	+	-	+
216VDQKADG	+	+	+	+	+	+	+	+
143NYMTGRT	-	+	+	+	+	+	+	+
47TNGDTSS	-	+	+	+	+	+	+	+
24EIYNKNG	-	+	+	+	+	+	-	+

-, Negative; +, Positive

Table 2) Antigenicity prediction of the investigated proteins (EMBOSS server)

Protein	Predicted Antigenic Region	Maximum Score pos*
Type 1 fimbria	30 GGTVHFKGEVVNAACAVDAG	43
	121 NVGVQIL	125
	106 TSVLALQNS	109
	4 KIFVIAAMSALSLSSAAALA	6
	52 DQTVQLGQVRSAKLATA	57
	72 SSAVGFNIQLDDCDTSVATKASVAFSGT	91
	153 IIPFQARYYAIGAA	163
	132 TPLALDGA	136
Type 3 fimbria	22 ASCTRLSSPTVMLDMVVGRVVVPPDLPVGSVILT	41
	4 RKLLTLFIVLMAL	9
	172 LETYLSANAITVVSPSCSVLSG	187
	112 TVNIVYPDVFSSRVY	118
	299 YITIPLHARFYQYG	302
	270 IGIQVLKDGSPLQFN	273
	80 AKIVSPGA	81
	222 IDLQCSGG	227
	138 FTLQIIKT	141
OmpA	288 AQSVVDYPVAKGI	294
	5 AIAIAVALAGFATVAQAA	9
	263 GSAVVLGYT	267
	206 AAPVVAPAPAPAPEVATKHFTLKSDVLFN	210
	326 ARAALIDCLAPD	332
	194 SLGVSYR	196
	347 YKEVVTQ	353
	147 DTGVSPVFAGGVEWA	152
	100 AQGVQLTAKLGYP	106
	68 GYRVNPYLG	72
	247 ALDQLYTQ	249
	116 DLDIYTR	120
OmpK35	6ILAVVIPALLVAGA	13
	268FEAVVQYQFDFGLRPSIGYVQT	273
	305ADLVKYIEVGTW	310
	350QAAVGIV	354
	325 VYAAYKFN	328
	161FGLVDGLSFALQYQ	170
	241IYAAVMYS	244
	118GAIYDVEA	120
	202GIALSAGY	206
	70NDQLIGY	75

* Maximum score is predicted at this amino acid

amino acids in favored regions (94.9% for type 1 fimbria, 98.36% for type 3 fimbria, 92.18% for OmpA, and 94.63% for OmpK35, respectively). In addition, GMQE (Global Model Quality Estimate) values for type 1 and 3 fimbriae, OmpA, and OmpK35 were as follows: 0.74, 0.90, 0.79, and 0.92, respectively. GMQE is coverage-dependent, a score below 0.5 means that half of the target is probably covered. To obtain discontinuous B cell epitopes, the PDB file of each protein was submitted to two independent servers, including Discotope and Ellipro servers. The results of each tool are shown in Table 3. The scores and regions predicted by each tool were compared, and the final epitopes were selected.

Toxicity, human similarity, allergenicity, and final epitope selection: The outputs of all servers including IEDB, ABCpred, EMBOSS, Expasy, Ellipro, and Discotope were compared. The sequences of the final select-

Table 3) Discontinuous (conformational) epitope prediction results

Protein	Prediction Sever	Epitope Number	Selected Epitopes
Type 1 fimbria	Discotope 2 Ellipro	0 2	N24, T25, T26, T27, N29- G58, Q59, V60, R61, S62, A63, K64, L65, A66, T67, A68, G69, S70-L111, Q112, N113, S114, A115, A116, G117, S118, A119, T120, N121- I163, G164, A165, A166, T167, A168, G169, T170
Type 3 fimbria	Discotope 2 Ellipro	1 5	M1, S2, L3, R4, K5, L6, L7, T8, L9, F10, I11, V12, L13, M14, A15, L16, G17, T18, T19, S20, S21, W22, A23,:S24, T26, R27, L28, S29, S30, P31, T32, V33, M34, D36, M37, V38, V39- T60, M61, S62, A63, P64, G65, G66, A67, S68, R70, C71, T72, S73, G74, T75- S123, R124, V125, Y126, N127, T128, A:T129, N130, Y131, S132,:L133,:E134, G135, S136- T153, G167, G168, N169, P170,:I171, Y175, S177, A178, N179, A180
OmpA	Discotope 2 Ellipro	6 5	K228, S229, D230, V231, L232, F233, N234, F235, N236, K237, A238, T239, L240, K241, P242, E243, G244, Q245, Q246, A247, L248, D249, Q250, L251, Y252, T253, Q254, L255, S256, N257, M258, D259, P260, K261,:D262, G263- T271, D272, R273, I274, G275, S276, E277, A278, Y279, N280, Q281, Q282, L283, S284, E285, K286, R287, Q289, A:S290, V292, D293, Y294, P295, V296, A297, K298, G299, I300, A:P301, A302, G303, K304, I305-, G309, M310, G311, E312, S313, N314, A:P315, V316, T317, G318, N319, T320, C321, D322, A:N323, V324, K325- A328, A329, L330, I331, A:D332, C333, L334, A335, P336, D337, R338
OmpK35	Discotope 2 Ellipro	13 2	Y274, Q275, F276, D77, F278, G279, L280-V345, A346, T347, D348, D349, Q350, A351, A352, V353, G354, I355,

ed epitopes are shown in Table 4. The toxicity and allergenicity of the selected epitopes were negative (data not shown). In addition, based on Blastp results, no significant similarity was observed between the selected epitopes and human antigens (> 90% similarity in identity and coverage). The antigenicity of each final epitope was also confirmed by the Antigenic Peptide Prediction server (http://imed.med.ucm.es/Tools/index.html).

Molecular characteristics of the final peptides: The instability index and aliphatic index were estimated to be -1.23 and 84.09 for type 1 fimbria, 50.97 and 124.64 for type 3 fimbria, 52.52 and 89.17 for OmpA, and 24.60 and 78.57 for OmpK35, respectively, indicating that the peptides were stable, unstable, unstable, and stable, respectively. In addition, the half-life of the selected peptides for type 1 and 3 fimbriae, as well as OmpA and OmpK35 in mammalian reticulocytes, yeast cells, and E. coli was estimated to be 100, >20, and>10 hours, 7.2, >20, and>10 hours, 4.4, >20, and>10 hours, 100, >20, and>10 hours, respectively, indicating long-term bioavailability. Furthermore, isoelectric pH (pI) was estimated as 5.32 for type 1 fimbria predicted peptide, 5.63 for type 3 fimbria predicted peptide, 5.88 for OmpA predicted peptide, and 8.47 for OmpK35 predicted peptide. The 3D structure of the final peptides is shown in Figure 1.

T-cell epitope (Class I and II MHC) prediction: The results of binding ability of the final epitopes to MHC class I and II have been illustrated in Table 5. The servers used successfully recognized the final epitopes;

Table 4) Sequences of the final B-cell epitopes predicted

Protein	Selected final epitope
Type 1 fimbria	Final epitope: VNGGTVHFKGEVVNAACAVDAG And DQTVQLGQVRSAKLATA Linear epitope: VNGGTVH and DQTVQLG* Discontinuous epitope: GQVRSAKLATAG** Antigenic region: GGTVHFKGEVVNAACAVDAG and DQTVQLGQVRSAKLATA ***
Type 3 fimbria	Final Epitope: TRLSSPTVMLDMVVGRVVVPPDLPVGSV Linear epitope: RLSSPTV * Discontinuous epitope: MSLRKLLTLFIVLMALGTTSSWASCTRLSSPTVMLDMVV** Antigenic region: ASCTRLSSPTVMLDMVVGRVVVPPDLPVGSVILT ***
OmpA	Final Epitope: AQSVVDYPVAKG Linear epitope: DYPVAKG * Discontinuous epitope: TDRIGSEAYNQQLSEKRQSVDYPVAKGIAPAGKI** Antigenic region: AQSVVDYPVAKGI ***
OmpK35	Final Epitope: VVQYQFDFGLRPSIGYVQTKG Linear epitope GYVQTKG * Discontinuous epitope: YQFDFGL** Antigenic region: FEAVVQYQFDFGLRPSIGYVQT ***

*Predicted by IEDB and ABCpred servers, **Predicted by Discotope/Ellipro severs, ***Predicted by EMBOSS

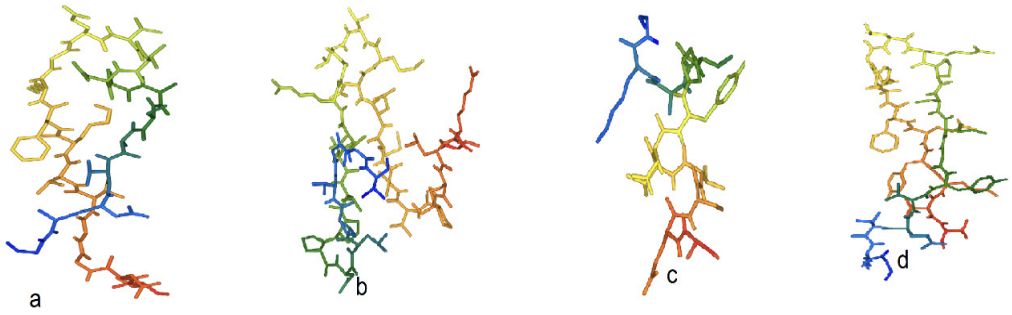

Figure 1) 3D structure of the final peptides predicted by PEP-FOLD 4: a) type 1 fimbria, b) type 3 fimbria, c) OmpA (c), and d) OmpK35. Colors show amino acids.

Table 5) Binding ability of the final peptides to class I and II MHC

Epitope	Predicted by HLA -I (Class I)	Predicted by HLA -II (class II)
VNGGTVHFKGEVVNAA*CAVDAG (Type 1 fimbriae)	HLA-B*08:01	HLA-DRB1*15:01 HLA-DRB1*07:01
TRLSSPTVMLDMVVGRVVVPPDLPVGSV (Type 3 fimbriae)	HLA-A*02:01 HLA-B*15:01	HLA-DRB1*03:01
AQSVVDYPVAKG (OmpA)	HLA-A*11:01 HLA-A*03:01 HLA-B*51:01	NA**
VVQYQFDFGLRPSIGYVQTKG (OmpK35)	HLA-A*02:01 HLA-B*35:01	HLA-DRB1*07:01

*Underlined region represents the specific sequence of class I and II MHC binding ability
**Not applicable

therefore, the activation of T cells could be expected by the predicted epitopes.

Discussion

Vaccination is considered a safe, simple, and impactful health intervention for reducing disease spread and preventing poor patient outcomes and even deaths from vaccine-preventable diseases [8, 34].

Different antigens such as lipopolysaccharide (LPS) and capsular antigens have been evaluated as potential candidates for vaccine targets; however, due to the toxic side effects of LPS and the genetic diversity of capsular antigens among *K. pneumoniae* strains, their use encounters serious limitations [35].

The present study focused on immunogenic proteins including type 1 and 3 fimbriae, OmpA, and OmpK35 to design a hypothetical vaccine against *K. pneumoniae* using bioinformatics tools.

Surface proteins such as fimbriae and OMPs have been reported to be appropriate candidates for vaccine development because they are involved in many biological processes including binding to host cells and activating the immune system and signaling pathways [36-38].

Based on this study results, 25 linear B-cell epitopes were identified: five in type 1 fimbria, eight in type 3 fimbria, seven in OmpA, and five in OmpK35. These predicted peptides could play a significant role in antibody-epitope interactions and must be presented in the final designed vaccine.

In type 1 fimbria, VNGGTVH and DQTVQLG epitopes were selected as the best potential vaccine candidates. These epitopes were located in 28-34 and 52-58 regions and as linear epitopes could interact with antibodies. In type 3 fimbria, the most successful epitope was RLSSPTV, this linear epitope, located in regions 27-33, was also successfully predicted by conformational epitope prediction servers, indicating the importance of this region as an epitope. In addition, linear epitopes and conformational epitopes were successfully identified in other investigated proteins (OmpA and OmpK35) (Table 4).

In this study, the SWISS-MODEL server was used to build the 3D structure of each protein.

The QMEANDisCo and GMQE scores revealed that the models were built appropriately (except for type 3 fimbria).

To achieve the best vaccine candidate, different bioinformatics tools must be applied. The results revealed that the predicted epitopes were appropriate because different independent servers predicted them.

In a study by Zhang et al. (2021) [8], different OMPs of *K. pneumoniae* were investigated. They reported that OMPs could successfully stimulate the immune system and therefore could be considered as appropriate candidates for vaccine development.

The physicochemical properties of epitopes are pivotal and must be considered when designing vaccines. For example, instability index, isoelectric pH, and peptide half-life could affect vaccine function [39, 40]. The results revealed that the final predicted epitopes in type 1 fimbria and OmpK35 were stable with instability indices of -1.23 and 24.6, respectively. In addition, the final predicted epitopes in type 1 and 3 fimbriae, OmpA, and OmpK35 showed long-term bioavailability (high estimated half-life), facilitating their use as a potential vaccine candidate.

The isoelectric point (pI) is known to be the pH at which a molecule is electrically neutral and carries no net electrical charge [39, 40]. The physicochemical pH of the human body ranges between 7.35 to 7.45; therefore, to avoid precipitation of the predicted peptide, the pI should not be close to the pH of the human body. The findings revealed that the pI value of the selected epitopes was far from the physicochemical pH of the human body.

The aliphatic index is another physicochemical property that shows stability against temperature. The results demonstrated that the predicted peptides were thermo-stable with aliphatic indices of 84.09, 124.64, 89.17, and 78.57 for type 1 fimbria, type 3 fimbria, OmpA, and OmpK35, respectively. To select the best vaccine candidate peptide, the allergenicity, toxicity, and human similarity of the predicted peptides must be investigated. Noteworthy, in this study, allergenicity, toxicity, and human similarity were negative for the predicted epitopes.

Some limitations must be considered, although appropriate peptides were identified based on bioinformatics tools, the effectiveness of the predicted peptides must also be investigated in vitro and in vivo (by cell culture method and animal model). In addition, other aspects of immune system responses such as interaction with MHC proteins and production of chemokines must be studied.

Conclusion

Overall, several bioinformatics tools were used to investigate four important surface proteins of *K. pneumoniae.* The present study results introduced four reliable B-cell and T-cell epitopes (each for one investigated protein) with appropriate physicochemical characteristics. The reported epitopes could be used to develop an effective vaccine against *K. pneumoniae*; however, further studies (in silico, in vitro, and in vivo) are recommended.

Acknowledgments

The authors would like to thank Dr. L. Shahraki for supporting this project.

Ethical approval: Not applicable.

Authors' contributions: HV: conceptualization, data collection, formal analysis, project administration, writing of the original draft; HV and VVa: methodology, software works.

Conflicts of interests: Non declared by authors.

Fundings: Non declared by authors.

Consent to participate: Not required

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