Exploring YidR Gene from *Klebsiella pneumoniae* To Design a Multi-epitope Vaccine Using Immunoinformatics Approach

Maulana Wildan Seputra[1*], Hani Susiati[2,] Tri Yudani Mardining Raras[3]

[1]Biomedical Science Post Graduate Programs, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[2]Department of Clinical Pathology, Saiful Anwar Hospital, Malang, Indonesia
[3]Department of Biochemistry, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

Abstract

Klebsiella pneumoniae is the main leading cause of hospital-acquired infection, leading to severe infections such as pneumonia and urinary tract infection. There is an urge to develop an efficient *Klebsiella* vaccine, and epitope-based vaccines may offer a practical choice. Therefore, this study aims to design an epitope-based vaccine construct. YidR gene is expressed in all strains of *Klebsiella* with 97.6% of sequence homology, which makes it a potential vaccine candidate. *In silico* approach was used to make a multi-epitope vaccine construct consisting of immunogenic epitopes against the YidR gene of *Klebsiella*. Acquired multi-epitopes vaccine was analyzed for antigenicity, allergenicity, toxicity, and various physicochemical parameters. Molecular docking simulation was performed using TLR2 and TLR4 to asses binding affinity with the designed vaccine construct. The results showed that the predicted multi-epitope-based vaccine candidate is non-allergen, non-toxic, and has an efficient binding affinity towards TLR2 and TLR4 that could effectively induce immune responses.

Keywords: *In Silico*, *Klebsiella pneumoniae*, vaccine design, YidR gene.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic, gram-negative bacterium and a common etiology in severe infections, such as pneumonia and vascular infection, which increase the healthcare-associated infection burden [1]. It has also been demonstrated that it can infect intracellularly through bladder epithelial cells in urinary tract infections [2].

Due to mutation as a form of evolution, *K. pneumoniae* developed an ability to acquire new genetic material. Because of that, two strains of *K. pneumoniae* are classified as classic and hypervirulent. The difference between these two is that the hypervirulent one has a higher virulence than the classic one, enabling it to infect a healthy individual, cause many sites of infection, and spread metastatically [3].

The hypervirulent strain of *K. pneumonia* explicitly has a virulence plasmid that differentiates it from the classic strain [4]. This virulence plasmid contains an antimicrobial resistance gene that is acquired in some way:

1. Plasmid acquisition that encodes antimicrobial resistance gene [5];
2. Gene insertion to the plasmid [6];
3. The classic strain acquires virulence plasmid from the hypervirulent strain [7].

*Correspondence Address:
Maulana Wildan Seputra
E-mail : maulanawildans@gmail.com
Address : Faculty of Medicine, Universitas Brawijaya, Veteran Malang, 65145.

The antimicrobial resistance of *K. pneumonia* is increasing steadily, thus making the therapy given more difficult than usual. Antibiotic therapy may not be effective because there is an increase in infection prevalence of drug-resistant strains or unwanted reactions from high-dosing antibiotic usage [8].

A vaccine is one measure to control infection by introducing epitopes of an antigen to the immune system. Epitopes are a small part of an antigen that can induce immune response through cell-mediated or antibody-mediated immunity. The whole system is required to acquire immunity [9].

Despite the alternative way to control infection, until now, no *Klebsiella* vaccine has been available, partly due to complicated production processes and high production costs [8]. Following those difficulties, *Klebsiella* is well-known for its strain's heterogenicity, which complicates the matter more [8].

YidR gene is found in all isolates acquired from bovine and human samples (308/308, 100%) [10]. This gene shared high homology (97.6%) across different isolates. This prevalence and similarity made this gene a potential antigen candidate for *K. pneumoniae* [10]. YidR gene is predicted to encode ATP/GTP-binding protein which appears to facilitate hyper-adherence phenotype for biofilm production in *Salmonella enterica* [8].

Using *In Silico* approach, researchers can develop a vaccine design with a rational and effective construct while still being reasonable in time need and production cost. This approach has several advantages, such as minimizing the unwanted immune responses, like allergenicity and toxic properties it may carry. With a more specific vaccine design, the vaccine can be designed with desired immunity response and is cost-effective [11,12]. In this study, we analyzed the potential of the YidR gene from *K. pneumonia* as an epitope-based vaccine through epitope prediction, physiochemical parameters analysis, and molecular docking.

MATERIAL AND METHOD

Protein Selection and Sequence Retrieval for Epitope Prediction

The previous iteration of the YidR gene from Rodrigues *et al.* [8] was used in this study. The obtained sequence was then submitted to NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi;accessed in April 2022) to acquire the FASTA sequence of YidR. The sequence with the highest query cover and percent identity is then chosen to be used in this study [13].

HLA Population Selection

Due to the vast amount of HLA in human populations, this study limits the population target to Indonesia. Using Allele Frequency Net Database (allelefrequencies.net; accessed April 2022) [14], the HLA allele with more than 20% of the population was selected. The chosen HLAs are listed in the Table 1 below.

Table 1. HLA populations in Indonesia chosen for this study

Allele	Population	% of Individuals with the allele
A*11:01	Indonesian Java Western	30.1
A*24:02	Indonesian Java Western	25.8
A*24:07	Indonesian Java Western	39.4
A*33:03	Indonesian Java Western	29.7
B*15:02	Indonesian Java Western	22.9
B*25:13	Indonesian Java Western	21.6
DRB1*07:01	Indonesian Java Western	25.4
DRB1*12:02	Indonesian Java Western	59.7
DRB1*15:02	Indonesian Java Western	41.1

Cytotoxic T-Cell Epitope Prediction

Cytotoxic T-Cell recognizes epitopes presented by HLA Class I on the surface of the antigen-presenting cells. Epitopes for Cytotoxic T-cell is predicted using NetMHCpan EL 4.1. (https://www.iedb.org/; accessed April 2022) as the method. Seven HLAs from the previous step were selected, which are HLA-A*11:01, HLA-A*24:02, HLA-A*24:07, HLA-B*15:02, HLA-B*15:13, HLA-B*15:21, and HLA-A*33:03 as our prediction target. The results are considered a good/suitable binder if the result is under 1% of the percentile rank. Later on, the predicted epitopes were evaluated [15] for (all accessed in April 2022):

1. Immunogenicity with IEDB https://www.iedb.org/
2. Antigenicity with VaxiJen; http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html
3. Toxicity with ToxinPred https://webs.iiitd.edu.in/raghava/toxinpred/design.php
4. Allergenicity with AllerTOP https://www.ddg-pharmfac.net/AllerTOP/method.html.

Helper T-Cell Epitope Prediction

Helper T-cell recognizes epitopes presented by HLA Class II on the surface of the antigen-presenting cells. Epitopes for T cell helper are predicted using the consensus method (iedb.org). Three HLAs from the previous step, DRB1_07:01, DRB1_12:02, and DRB1_15:02, were tested for the prediction target. The results are considered a strong binder if the result is under 2% of the adjusted rank. Later on, the predicted epitopes were evaluated further [15] (all accessed in April 2022):

1. Antigenicity by VaxiJen: http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html
2. Toxicity by ToxinPred: https://webs.iiitd.edu.in/raghava/toxinpred/design.php
3. Allergenicity by AllerTOP: https://www.ddg-pharmfac.net/AllerTOP/method.html)
4. IFN induction by IFNepitope: https://webs.iiitd.edu.in/raghava/ifnepitope/predict.php
5. IL4 induction by IL4Pred: https://webs.iiitd.edu.in/raghava/il4pred/predict.php
6. IL10 induction by IL10Pred: https://webs.iiitd.edu.in/raghava/il10pred/predict3.php

B Cell Epitope Prediction

B cell has important role in producing immunity memory. Epitopes for B cell is predicted using BEPIPRED 2.0 method in IEDB (https://www.iedb.org/; accessed April 2022). The prediction epitopes were determined afterwards [15] (all accessed in April 2022):

1. Antigenicity with VaxiJen; http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html
2. Toxicity with ToxinPred https://webs.iiitd.edu.in/raghava/toxinpred/design.php
3. Allergenicity with AllerTOP https://www.ddg-pharmfac.net/AllerTOP/method.html.

Selection of Adjuvant and Protein Linker for Vaccine Construction

The adjuvant is a component used in a vaccine construct to enhance vaccine efficacy [16]. Cholera toxin subunit B was utilized in this study as an adjuvant because it increased immune response in mucosal tissues or systemic [15]. To connect all of the epitopes, we used linkers as described by Shey *et al*. [17], which are EAAAK linker to connect adjuvant with the whole sequence, AAY linker to connect epitopes from T cell cytotoxic, and GPGPG linker to connect epitopes from T cell helper and B cell. Structure for multi-epitope construct was generated using trRosetta in April 2022 with this link https://yanglab.nankai.edu.cn/trRosetta/ [18]. This vaccine's modeling was evaluated using Ramachandran's geometrical analysis (https://molprobity.biochem.duke.edu/index.php ; accessed April 2022) [19].

Evaluation of Physicochemical Properties of Vaccine Construct

All epitopes obtained from the prediction and vaccine construct were evaluated for physicochemical parameters. It includes molecular weight, instability index, aliphatic index, GRAVY, theoretical isoelectric point, and estimated half-life using ExPasy Protparam tools (http://web.expasy.org/protparam; accessed April 2022) [16].

Molecular Docking of Vaccine with TLR2 and TLR4

Toll-Like Receptor (TLR) is a transmembrane protein that functions in recognizing part of infecting pathogen to activate the immune response. In *K. pneumoniae* infection, TLR2 and TLR4 expression is elevated as a response [16]. Therefore, this study used TLR2 and TLR4 as receptors in molecular docking.

TLR2 and TLR4 protein structure was acquired using Protein Data Bank (www.rcsb.org) with a PDB number of 2Z7X for TLR2 and 4G8A for TLR4. All of the protein was purified from another ligand and other unusable molecules using PyMOL software. Molecular docking was performed using the HDOCK server (https://hdock.phys.hust.edu.cn/; accessed May 2022) [20] with reference to Pizzuto *et al.* [20] for TLR2 binding site and the previously acquired conformation for TLR4.

RESULT AND DISCUSSION

A total of 85 epitopes for T cell cytotoxic, 11 epitopes for T cell helper, and 14 epitopes for B cell were found (Supplementary 1–3). The acquired epitopes are then evaluated for a vaccine candidate's required characteristics (antigenicity, allergenicity, and toxicity). It was done to ensure that no untargeted response is triggered and to minimize the adverse effect of the vaccine (Table 2, Table 3, and Table 4) [21].

Table 2. Result of the evaluated epitopes used in this study

Epitope	Peptide	Antigenicity	Toxicity	Allergenicity
B-Cell	TFAPRHHQLTN	Antigen	Non-Toxin	Non-Allergen
B-Cell	HVLHQRDPALDLR	Antigen	Non-Toxin	Non-Allergen
B-Cell	PSGASFTGE	Antigen	Non-Toxin	Non-Allergen
Cytotoxic T-Cell	VTVHPTQER (HLA-A*11:01)	Antigen	Non-Toxin	Non-Allergen
Cytotoxic T-Cell	WQYDFHHRR (HLA-A*33:03)	Antigen	Non-Toxin	Non-Allergen
Helper T-Cell	GTVETIYRATQGAHV (HLA-DRB1*07:01)	Antigen	Non-Toxin	Non-Allergen
Helper T-Cell	VETIYRATQGAHVGV (HLA-DRB1*07:01)	Antigen	Non-Toxin	Non-Allergen

Table 3. Result of the immunogenicity evaluation of Cytotoxic T-Cell epitopes used in this study

Epitope	Peptide	Immunogenicity
Cytotoxic T-Cell	VTVHPTQER	0.29415
Cytotoxic T-Cell	WQYDFHHRR	0.28446

Table 4. Result of the cytokines induction evaluation of Helper T-Cell epitopes used in this study

Epitope	Peptide	IFNepitope	IL4	IL10
Helper T-Cell	GTVETIYRATQGAHV	Negative	Inducer	Inducer
Helper T-Cell	VETIYRATQGAHVGV	Positive	Inducer	Inducer

ISSN. 2087-2852
E-ISSN. 2338-1655

Two cytotoxic T-cell epitopes were chosen based on the highest immunogenicity score (Table 3). These two epitopes are presented by HLA-A*33:03, covering 29.7% of the Indonesian population (Table 1). Two helper T-cell epitopes were chosen despite one having a negative IFNgamma score, and both are positive for inducing IL-4 and IL-10 (Table 4). Both T-helper epitopes are presented by HLA-DRB1*07:01, which is one of the major HLA Class II alleles, covering 25.4% of the Indonesian population (Table 1). Despite their ability to induce IL-4, which associated with the allergic reaction, the epitopes are still chosen since they are promising/suitable antigens, non-toxin, and non-allergen (Table 2).

Then, a construct was designed based on Shey *et al.* [17] with Cholera Toxin subunit B (CTB) as an adjuvant (Fig. 1). Adjuvant is required to raise the efficacy of the vaccine [16]. At the same time, CTB is selected because of its properties to increase immunogenicity and augment immune response in mucosal tissues and systemic [15]. According to Shey *et al.* [17], the vaccine construct can be connected with these linkers:

1. Adjuvant is located in the N-terminal of the sequence, and connected with the rest using EAAAK linker.
2. Epitope sequences from T cell cytotoxic are connected using AAY linker.
3. Epitope sequences from T cell helper and B cell are connected using GPGPG linker.

The trRosetta server was employed for the 3D modeling of the vaccine construct (https://yanglab.nankai.edu.cn/trRosetta/). A Template Modelling (TM) score of 0.576 was obtained for the model predicted, which was higher than 0.5, indicating that the model predicted has high confidence in topology prediction [18].

Next, the model was evaluated with the help of Ramachandran geometry analysis. This tool verified the model using indirect stereochemical properties and the geometrical complex of an amino acid according to electrostatic rules [19]. It showed that the vaccine construct has 98.82% in 'Favored rotamers' and 94.42% in 'Ramachandran favored. The number is over 90%, implying that the amino acid position in the 3D model is in stable and correct position [19]. There were also 3 Ramachandran outliers in Asp59, Ile140, and Gln163 (Table 6, Fig. 2).

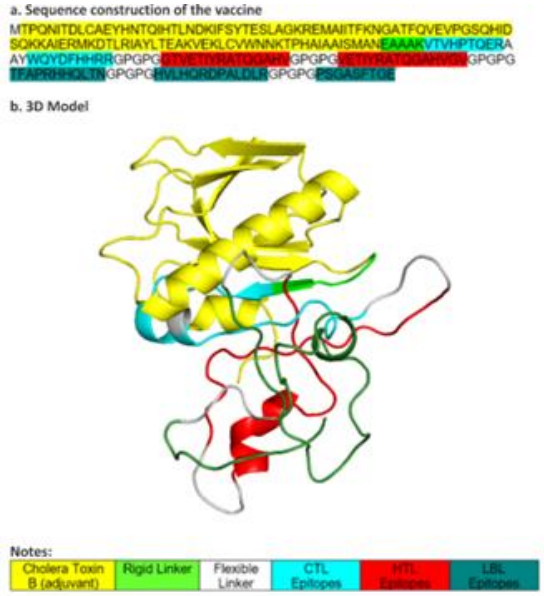

Figure 1. Vaccine Construction;
a) Sequences construction of the vaccine;
b) 3D model acquired from trRosetta server

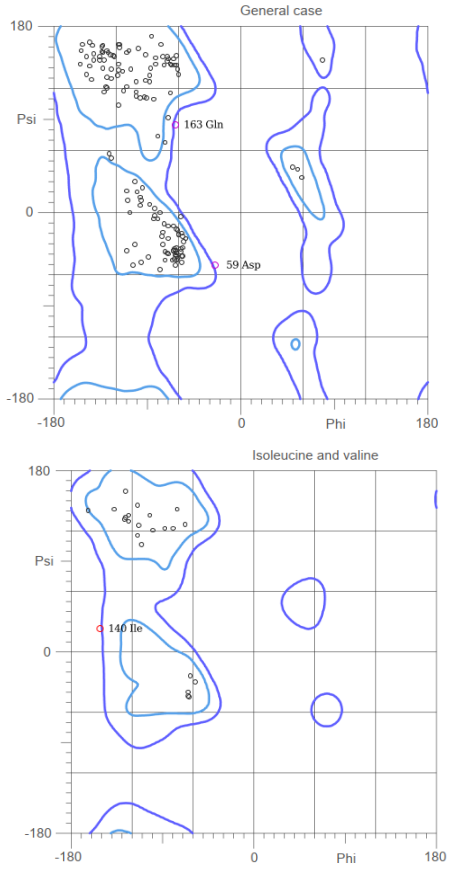

Figure 2. Ramachandran plot analysis from the vaccine model

The physiochemical parameters of this vaccine were also observed using ExPasy Protparam tools. The result is presented below (Table 5, Supplementary 4).

ISSN. 2087-2852
E-ISSN. 2338-1655

Table 5. Physicochemical properties of the vaccine

Length	Molecular Weight	Theoretical pI	Instability Index
225	24532.47	8.54	22.48
Aliphatic Index	**GRAVY**	**Estimated Half Life**	
63.82	-0.620	30 hours (mammalian reticulocytes, in vitro)	

TLR2 and TLR4 are transmembrane proteins that recognize part of pathogens to activate the immune system. In *K. pneumoniae* infection, TLR2 and TLR4 expression is elevated in epithelial tissue [16]. Within the myeloid differentiation primary response gene (MyD88) signaling pathway, TLR2, and TLR4 are prominent for many cytokines' excretion, including TNF-α and Interleukin-1, Interleukin-6 and Interleukin-8 which are essential as a chemoattractant for neutrophils, also NF-κB which have roles in inflammation [22].

Molecular docking was performed as a reference to determine the binding affinity of the vaccine to TLR2 and TLR4. TLR2 and TLR4 structure data was accessed from Protein Data Bank (www.rcsb.org) with PDB numbers 2Z7X and 4G8A. The obtained protein structure was then processed using PyMOL software to obtain pure TLR2 and TLR4 protein.

According to Pizzuto *et al.* [20], TLR2 has a binding site listed specifically in the main chain, which are L324, F325, F349, L350, S329, and the side chain, which is N294. For TLR4, we used a specific binding site from the Protein Data Bank crystal structure, which is already bound to a complex of TLR4 and bacterial lipopolysaccharide.

The HDOCK server was employed for the molecular docking of our vaccine construct. HDOCK is an integrated place for homology search, template-based modeling, structure prediction, macromolecular docking, and other information with an accurate and fast docking result [23]. Surprisingly our vaccine constructs binding affinity scores of -244,72 and -266,51 as docked with TLR2 and TLR4 subsequently (Table 6, Fig. 3, Fig. 4).

Table 6. Binding affinity score from molecular docking results

Receptor	TM-Score	R-Outlier	R-Favored (%)	Z-Score	Binding Affinity Score
TLR2	0.576	3	94.42%	-0.42	-244.72
TLR4	0.576	3	94.42%	-0.42	-266.51

Notes
R-Outlier = Ramachandran Outlier
R-Favored = Ramachandran Favored

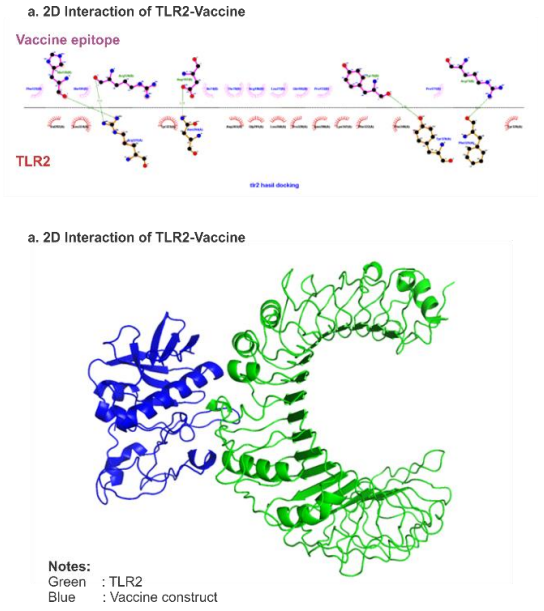

Figure 3. Molecular docking results of TLR2 and vaccine construct

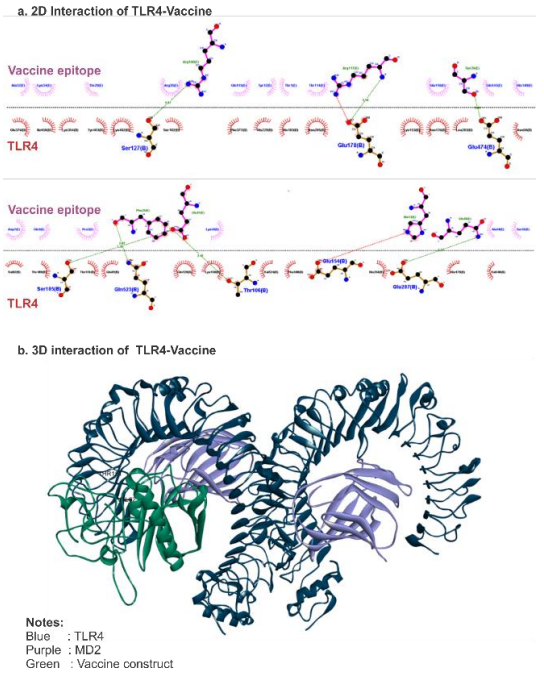

Figure 4. Molecular docking results of TLR4 and vaccine construct

Binding affinity is scored from conformational results from docking, which has the lowest energy. It represents the similarity of the result with the crystallography structure [24]. Negative results mean hydrogen bonding and van der walls are the most prominent interactions between protein and ligand complex stabilization, suggesting the binding process is spontaneous [25]. With a binding affinity score of -244,72 and -266,51 for TLR2 and TLR4, respectively, the

ISSN. 2087-2852
E-ISSN. 2338-1655

vaccine construct is able to bind effectively to TLR2 and TLR4.

Despite that, this study is limited and needs further study before this vaccine construct is clinically approved. This study uses using *in silico* approach, thus making this study only a prediction-based analysis using simulations and predictions from computational algorithms and data banks from past studies. This study is yet unable to represent actual protein properties when expressed in laboratory conditions and needs further study for clinical approval.

CONCLUSION

The present study shows an *in-silico* approach to designing vaccines against *Klebsiella pneumoniae*. Multi-epitope-based vaccine constructs based on epitopes for T cells and B cells using the YidR gene demonstrated that the vaccine constructs could bind to TLR2 and TLR4 effectively. This study could be beneficial in delivering information on the epitope-based construct's potential as a protective approach for immunization against bacterial infection.

REFERENCES

[1] Magill, S.S., J.R. Edwards, W. Bamberg, Z.G. Beldavs, G. Dumyati, M.A. Kainer, *et al.* 2014. Multistate point-prevalence survey of health care–associated infections. *N. Engl. J. Med.* 370. 1198–1208. DOI: 10.1056/NEJMoa1306801.

[2] Rosen, D.A., J.S. Pinkner, J.M. Jones, J.N. Walker, S. Clegg, S.J. Hultgren. 2008. Utilization of an intracellular bacterial community pathway in *Klebsiella pneumoniae* urinary tract infection and the effects of FimK on type 1 pilus expression. *Infect Immun.* 76(7). 3337-45. DOI: 10.1128/IAI.00090-08.

[3] Russo, T.A., C.M. Marr. 2019. Hypervirulent *Klebsiella pneumoniae*. *Clin. Microbiol. Rev.* 32(3). e00001-19.

[4] Russo, T.A., R. Olson, C.T. Fang, N. Stoesser, M. Miller, U. MacDonald, A. Hutson, J.H. Barker, R.M. La Hoz, J.R. Johnson. 2018. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. *J. Clin. Microbiol.* 56. e00776-18.

[5] Feng, Y., Y. Lu, Z. Yao, Z. Zong. 2018. Carbapenem-resistant hypervirulent *Klebsiella pneumoniae* of sequence type 36. *Antimicrob. Agents Chemother.* 62. e02644-17.

[6] Zhang, R., D. Lin, E.W. Chan, D. Gu, G.X. Chen, S. Chen. 2016. Emergence of carbapenem-resistant serotype K1 hypervirulent *Klebsiella* pneumonia strains in China. *Antimicrob. Agents. Chemother.* 60. 709 –711.

[7] Gu, D., N. Dong, Z. Zheng, D. Lin, M. Huang, L. Wang, E.W. Chan, L. Shu, J. Yu, R. Zhang, S. Chen. 2017. A fatal outbreak of ST11 carbapenemresistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect. Dis.* 18. 37– 46.

[8] Rodrigues, M.X., Y. Yang, E.B.Jr. Meira de Souza, J. do Carmo Silva, R.C. Bicalho. 2020. Development and evaluation of a new recombinant protein vaccine (YidR) against *Klebsiella pneumoniae* infection. *Vaccine*. 38(29). 4640–4648. DOI: 10.1016/j.vaccine.2020.03.057.

[9] Clem, A.S. 2011. Fundamentals of vaccine immunology. *J. Glob. Infect. Dis*. 3(1). 73–78. DOI: 10.4103/0974-777X.77299.

[10] Yang, Y., C.H. Higgins, I. Rehman, K.N. Galvao, I.L. Brito, M.L. Bicalho, *et al.* 2019. Genomic diversity, virulence, and antimicrobial resistance of *Klebsiella pneumoniae* strains from cows and humans. *Appl. Environ. Microbiol.* 85(6). e02654-18. DOI: 10.1128/AEM.02654-18.

[11] Kazi, A., C. Chuah, A.B.A. Majeed, C.H. Leow, B.H. Lim, C.Y. Leow. 2018. Current progress of immunoinformatics approach harnessed for cellular- and antibody-dependent vaccine design. *Pathog. Glob. Health.* 112(3), 123-131. DOI: 10.1080/20477724.2018.1446773.

[12] Parvizpour, S., M.M. Pourseif, J. Razmara, M.A. Rafi, Y. Omidi. 2020. Epitope-based vaccine design: a comprehensive overview of bioinformatics approaches. *Drug Discov. Today.* 25(6). 1034-1042. DOI: 10.1016/j.drudis.2020.03.006.

[13] Madden T. 2002. The BLAST Sequence Analysis Tool. In: McEntyre, J., J. Ostell (Eds). The NCBI Handbook. Bethesda (MD): National Center for Biotechnology Information (US), Chapter 16. Available at: http://www.ncbi.nlm.nih.gov/books/NBK21097/.

[14] Gonzalez-Galarza, F.F., A. McCabe, E.J. Santos, J. Jones, L.Y. Takeshita, N.D. Ortega-Rivera, G.M. Del Cid-Pavon, K. Ramsbottom, G.S. Ghattaoraya, A. Alfirevic, D. Middleton, A.R. Jones. 2020. Allele frequency net

database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acid Res.* 48. D783-8.

[15] Sanami, S., M. Alizadeh, M. Nosrati, K.A. Dehkordi, F. Azadegan-Dehkordi, S. Tahmasebian, *et al.* 2021. Exploring SARS-COV-2 structural proteins to design a multi-epitope vaccine using immunoinformatics approach: an in silico study. *Comput. Biol. Med*. 133. 104390. DOI: 10.1016/ j.compbiomed.2021.104390.

[16] Rahmat Ullah, S., M. Majid, M.I. Rashid, K. Mehmood, S. Andleeb. 2021. Immunoinformatics driven prediction of multiepitopic vaccine against *Klebsiella pneumonia* and *Mycobacterium tuberculosis* coinfection and its validation via *In Silico* expression. *Int. J. Pept. Res. Ther.* 27. 987–999. DOI: 10.1007/s10989-020-10144-1.

[17] Shey, R.A., S.M. Ghogomu, K.K. Esoh, N.D. Nebangwa, C.M. Shintouo, N.F. Nongley*et al.* 2019. In-silico design of a multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. *Sci. Rep*. 9(1), 1-18.

[18] Du, Z., H. Su, W. Wang, L. Ye, H. Wei, Z. Peng, *et al.* 2021. The trRosetta server for fast and accurate protein structure prediction. *Nat. Protoc*. 16(12). 5634-5651.

[19] Patil, V.M., K. Balasubramanian, N. Masand. 2019. Dengue virus polymerase: a crucial target for antiviral drug discovery. In: Viral Polymerases. Elsevier. 387-428. DOI: 10.1016/B978-0-12-815422-9.00014-0.

[20] Pizzuto, M., M. Gangloff, D. Scherman, N.J. Gay, V. Escriou, J.M. Ruysschaert, C. Lonez. 2017. Toll-like receptor 2 promiscuity is responsible for the immunostimulatory activity of nucleic acid nanocarriers. *J. Control. Release*. 247. 182-193.

[21] Zepp, F. 2010. Principles of vaccine design—lessons from nature. *Vaccine*. 28. C14-C24.

[22] Kawasaki, T., T. Kawai. 2014. Toll-Like Receptor Signaling Pathways. *Front. Immunol.* 5. 461. DOI: 10.3389/fimmu.2014. 00461.

[23] Yan, Y., H. Tao, J. He, S.Y. Huang. 2020. The HDOCK server for integrated protein-protein docking. *Nat. Protoc.* 15(5). 1829–1852. DOI: 10.1038/s41596-020-0312-x.

[24] Morris, G.M., M. Lim-Wilby. 2008. Molecular docking. *Methods Mol. Biol.* 443. 365-382. DOI: 10.1007/978-1-59745-177-2_19.

[25] Qureshi, M.A., S. Javed. 2022. Investigating binding dynamics of trans resveratrol to HSA for an efficient displacement of aflatoxin B1 using spectroscopy and molecular simulation. *Sci. Rep.* 12(1). 1-17.

SUPPLEMENTARY

Supplementary 1. Candidate Epitope MHC I

ALLELE	#	START	END	LENGTH	PEPTIDE	CORE	ICORE	SCORE	PERC. RANK	Immunogenicity	Antigenicity	Toxicity	Allergenicity
HLA-A*11:01	1	67	75	9	VTVHPTQER	VTVHPTQER	VTVHPTQER	0.554665	0.25	0.03243	Antigen	Non-Toxin	Non-Allergen
HLA-A*11:01	1	379	387	9	AIVFSPDGK	AIVFSPDGK	AIVFSPDGK	0.467985	0.33	-0.00182	Antigen	Non-Toxin	Non-Allergen
HLA-A*11:01	1	68	76	9	TVHPTQERY	TVHPTQERY	TVHPTQERY	0.237656	0.77	0.04284	Antigen	Non-Toxin	Non-Allergen
HLA-A*24:07	1	5	13	9	TFAPRHHQL	TFAPRHHQL	TFAPRHHQL	0.718941	0.04	0.04201	Antigen	Non-Toxin	Non-Allergen
HLA-A*33:03	1	67	75	9	VTVHPTQER	VTVHPTQER	VTVHPTQER	0.669339	0.13	0.29415	Antigen	Non-Toxin	Non-Allergen
HLA-A*33:03	1	89	97	9	WQYDFHHRR	WQYDFHHRR	WQYDFHHRR	0.571215	0.21	0.28446	Antigen	Non-Toxin	Non-Allergen
HLA-A*33:03	1	143	151	9	YNDHVLHQR	YNDHVLHQR	YNDHVLHQR	0.437298	0.39	0.13668	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	110	118	9	DAMDITPPY	DAMDITPPY	DAMDITPPY	0.899684	0.02	0.11562	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	68	76	9	TVHPTQERY	TVHPTQERY	TVHPTQERY	0.870672	0.03	0.04284	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	130	138	9	HVYSPNGQF	HVYSPNGQF	HVYSPNGQF	0.810082	0.05	-0.22364	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	149	157	9	HQRDPALDL	HQRDPALDL	HQRDPALDL	0.332884	0.49	0.06875	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	358	366	9	HAGTGDITF	HAGTGDITF	HAGTGDITF	0.261319	0.67	0.23894	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:02	1	266	274	9	GVSQRRLTF	GVSQRRLTF	GVSQRRLTF	0.210936	0.88	-0.05782	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	110	118	9	DAMDITPPY	DAMDITPPY	DAMDITPPY	0.371252	0.13	0.11562	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	70	78	9	HPTQERYVF	HPTQERYVF	HPTQERYVF	0.25244	0.3	0.06326	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	337	345	9	FNWHPSGEW	FNWHPSGEW	FNWHPSGEW	0.221636	0.35	0.02502	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	358	366	9	HAGTGDITF	HAGTGDITF	HAGTGDITF	0.152492	0.6	0.23894	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	266	274	9	GVSQRRLTF	GVSQRRLTF	GVSQRRLTF	0.116657	0.81	-0.05782	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	5	13	9	TFAPRHHQL	TFAPRHHQL	TFAPRHHQL	0.112322	0.85	0.04201	Antigen	Non-Toxin	Non-Allergen
HLA-B*15:13	1	149	157	9	HQRDPALDL	HQRDPALDL	HQRDPALDL	0.105504	0.91	0.06875	Antigen	Non-Toxin	Non-Allergen

Notes: Epitope Candidate

Supplementary 2. Th cell (MHC II) properties

Peptide	adjusted rank	Antigenicity	Toxicity	Allergenicity	IFNepitope	IL4	IL10
ETIYRATQGAHVGVV	1.7	Probable Antigen	Non-Toxin	Allergen	Negative	Inducer	Inducer
TIYRATQGAHVGVVT	1.7	Probable Antigen	Non-Toxin	Allergen	Positive	Inducer	Inducer
GTVETIYRATQGAHV	1.8	Probable Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Inducer
VETIYRATQGAHVGV	1.8	Probable Antigen	Non-Toxin	Non-Allergen	Positive	Inducer	Inducer
FVSFTYNDHVLHERD	1.9	Probable Non-Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Non-Inducer
GQFVSFTYNDHVLHE	1.9	Probable Non-Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Non-Inducer
NGQFVSFTYNDHVLH	1.9	Probable Non-Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Non-Inducer
PNGQFVSFTYNDHVL	1.9	Probable Non-Antigen	Non-Toxin	Allergen	Negative	Inducer	Non-Inducer
QFVSFTYNDHVLHER	1.9	Probable Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Non-Inducer
SPNGQFVSFTYNDHV	1.9	Probable Non-Antigen	Non-Toxin	Allergen	Negative	Inducer	Non-Inducer
YSPNGQFVSFTYNDH	1.9	Probable Non-Antigen	Non-Toxin	Allergen	Negative	Inducer	Non-Inducer

Peptide	adjusted rank	Antigenicity	Toxicity	Allergenicity	IFNepitope	IL4	IL10
GTVETIYRATQGAHV	1.8	Probable Antigen	Non-Toxin	Non-Allergen	Negative	Inducer	Inducer
VETIYRATQGAHVGV	1.8	Probable Antigen	Non-Toxin	Non-Allergen	Positive	Inducer	Inducer

Notes:

Epitope Candidate

ISSN. 2087-2852
E-ISSN. 2338-1655

Supplementary 3. B cell properties

Start	End	Peptide	Length	Antigenicity	Toxicity	Allergenicity
5	15	TFAPRHHQLTN	11	Antigen	Non-Toxin	Non-Allergen
32	40	PSGASFTGE	9	Antigen	Non-Toxin	Non-Allergen
84	92	RPDAQWQYD	9	Antigen	Non-Toxin	Allergen
114	126	ITPPYTPGALRGG	13	Antigen	Non-Toxin	Allergen
146	158	HVLHQRDPALDLR	13	Antigen	Non-Toxin	Non-Allergen
167	180	GPVTPQGQHPREYG	14	Non-Antigen	Non-Toxin	Non-Allergen
191	214	TTPAPAPGSDEINRAYEEGWVGNH	24	Non-Antigen	Non-Toxin	Non-Allergen
220	229	GDTLAENGDK	10	Antigen	Non-Toxin	Allergen
238	254	LPQDEAGWKQPGGAPLA	17	Non-Antigen	Non-Toxin	Non-Allergen
274	289	FTHHRRYPGLVNVPRH	16	Non-Antigen	Non-Toxin	Allergen
320	333	GGEPRQLTHHASGI	14	Antigen	Non-Toxin	Allergen
367	377	LTDTHAHAPSA	11	Antigen	Non-Toxin	Allergen

Start	End	Peptide	Length	Antigenicity	Toxicity	Allergenicity
5	15	TFAPRHHQLTN	11	Antigen	Non-Toxin	Non-Allergen
32	40	PSGASFTGE	9	Antigen	Non-Toxin	Non-Allergen
146	158	HVLHQRDPALDLR	13	Antigen	Non-Toxin	Non-Allergen

Notes:

Epitope Candidate

Supplementary 4. Physico-chemical properties

Epitope	Peptide	Length	ID*	MW	II**	AI***	GRAVY	Theoretical pI	The estimated half-life
Cytotoxic T-Cell	VTVHPTQER	9	KP	1066.18	9.28	64.44	-1,033	6.72	100 hours (mammalian reticulocytes, in vitro)
Cytotoxic T-Cell	WQYDFHHRR	9	KP, PR	1344.46	82.09	0	-2,422	8.76	2.8 hours (mammalian reticulocytes, in vitro)
Helper T-Cell	VETIYRATQGAHVGV	15	KP, EC	1600.79	-23.95	97.33	0.1667	6.72	100 hours (mammalian reticulocytes, in vitro)
Helper T-Cell	GTVETIYRATQGAHV	15	KP, EC	1602.77	-23.95	78	-0.16	6.75	30 hours (mammalian reticulocytes, in vitro).
Continue B-Cell	TFAPRHHQLTN	11	KP, EB	1321.46	34.81	44.55	-1,136	9.47	20 hours (mammalian reticulocytes, in vitro)
Continue B-Cell	HVLHQRDPALDLR	13	KP, EB, SE	1569.79	38.86	120	-0.77	6.92	3.5 hours (mammalian reticulocytes, in vitro)
Continue B-Cell	PSGASFTGE	9	KP, SF, EC, EB	851.87	3.04	11.11	-0.4	4	5.5 hours (mammalian reticulocytes, in vitro)

Notes:
*Query Cover, Identity 100 %, **Instability Index**, ***Aliphatic Index,**
MW: Molecular Weight, KP: *Klebsiella pneumoniae*, PR: *Providencia rettgeri*, EC: *Escherichia coli*, EB: *Enterobacter*, SE: *Salmonella enterica*, SF: *Shigella flexneri*.