

## Prediction of promiscuous epitopes in NSP2 of Chikungunya virus: An *in-silico* approach

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**Abstract.** This study is focused towards developing a global consensus sequence of non-structural protein 2 (NSP2), a protease of Chikungunya Virus (CHIKV) and predict immunogenic promiscuous T-cell epitopes based on various bioinformatics tools. To date, no epitope data is available for the Chikungunya virus in the IEDB database. In this study, 100 available nucleotide sequences of NSP2-CHIKV belonging to different strains were downloaded from the National Centre for Biotechnology Information (NCBI) database. The nucleotide sequences were subjected to translated sequencing using the EXPASY tool followed by protein alignment using the CLC workbench and a global consensus sequence for the respective protein was developed. IEDB tool was used to predict HLA-I and HLA-II binding promiscuous epitopes from the consensus sequence of NSP2-CHIKV. Thirty-four B-cell based epitopes are predicted and the promiscuous epitope is VVDTTGSTKPDPGD at position 341-354. Twenty-six MHC-I short peptide epitopes are predicted to bind with HLA-A. The promiscuous epitopes predicted to bind with HLA-A\*01:01 are VTAIVSSLHY, SLSESATMVY, FSKPLVYY, QPTDHSVGEY at positions 317-326, 84-93, 535-544 and 15-24 with percentile ranks 0.17, 0.39, 0.51 and 0.81, respectively. Twenty-four MHC-II short peptide epitopes are predicted for HLA-DRB. The promiscuous epitope predicted to bind with HLA-DRB\*01:01 is VVGEYLVLPQTVLRS from 20-35 with a lowest percentile rank of 0.01. These predicted epitopes can be effective targets towards development of vaccine against CHIKV. Epitopes predicted in this study displayed good binding affinity, antigenicity and promiscuity for the HLA classes. These predicted epitopes can prove to be translationally important towards the development of CHIKV.

### INTRODUCTION

CHIKV is an alphavirus that causes arthralgia and is transmitted through mosquito bites. CHIKV is not fatal yet its symptoms can be severe and disturbing. These symptoms include fever, joint pain, muscle pain, joint swelling, headache, back pain, nausea, maculopapular rash on trunk and extremities of body. CHIKV was first isolated from human serum in Tanzania, 1952. In 2004 there was an outbreak of this disease, which spread through Africa, South East Asia and India with large sporadic epidemics (Waheed *et al.*, 2017). CHIKV is transmitted by *Aedes aegypti*

and *Aedes albopictus* (Petersen & Powers, 2016). Humans play the role of chief carriers and multipliers of the disease. CHIKV circulates in the blood stream of an infected individual and upon the bite of a naive mosquito (*Aedes aegypti*) on an infected individual, the mosquito will acquire the virus (Rausalu *et al.*, 2016). This infection is likely to take place in elderly individuals or patients with a suppressed immune response.

CHIKV is a positive single stranded RNA virus with a genome size of 12 kb. It encodes two polyproteins, the non-structural polyprotein which consists of four proteins (NSP1, NSP2, NSP3 and NSP4) and the

structural polyprotein, which consists of five proteins (E3, E2, 6K, E1 and Capsid) (Jain *et al.*, 2017; Solignat *et al.*, 2009; Thiboutot *et al.*, 2010). A previous study suggested that NSP2 is involved in the activation of the viral replication complex through its protease and helicase activity (Law *et al.*, 2019). In NSP2, the protease domain lies at the C-terminal domain (471-798) and the helicase domain at the N terminal (1-470) (Jadav *et al.*, 2012; Jain *et al.*, 2017). Replication and proliferation of CHIKV relies upon protease activity of the NSP2 protein because it cleaves the non-structural polyprotein (NSP1-4) replication complex into individual functional units. Furthermore, NSP2 assumes a significant role in host T-cell shut off, inhibition of antiviral reactions, enhanced virus gene expression and evasion host immune response (Bourai *et al.*, 2012; Jadav *et al.*, 2012).

CHIKV has infected more than 165,000 individuals in Asia, Africa and the Pacific Islands. Fifty-seven cases of CHIKV reported in US have been associated with traveling. The possibility of CHIKV outbreaks in the US is a real public health threat (Thiberville *et al.*, 2013; Yactayo *et al.*, 2016). In 2016, an outbreak of CHIKV occurred in Karachi, Pakistan. According to different medical sources, approximately 30,000 individuals were infected (Ali & Dasti, 2018). Since 2004 there have been massive outbreaks of this virus and there is no FDA approved vaccine available for this disease up till now. The possibility of CHIKV outbreaks all over the world is a real public health threat. This outbreak may get complicated with time due to poor sanitation systems, prolonged periods of warm weather, less effective mosquito eradication methods and stagnant water in different areas of the epidemic countries.

This study targets the protease/helicase protein of CHIKV for the prediction of promiscuous epitopes. Different available online tools were used for the future development of an effective and safe epitope-based vaccine against CHIKV.

## MATERIALS AND METHODS

In this study, NSP2-CHIKV, a protease and a helicase, was observed through *in-silico* tools. To analyze it, a phylogenetic tree and global consensus sequence for NSP2 was developed that was used for prediction of epitopes using various bioinformatics-based tools.

### Extraction of Sequences

One hundred homologous sequences of NSP2-CHIKV from different regions of the world were retrieved from NCBI. The nucleotide sequences were translated to amino acid sequences using EXPASY.

### Multiple Sequence Alignment

All amino acid sequences, along their accession numbers and country names, were fed into CLC main Workbench (version 8.1) using the default setting to develop multiple sequence alignment global consensus sequences and a phylogenetic tree.

### Epitope Prediction

B-cell and T-cell epitopes were predicted from global consensus sequences of NSP2-CHIKV using IEDB, an online bioinformatics tool. T-cell based immune epitopes against NSP2-CHIKV for multiple alleles of humans and for various species can be predicted using IEDB.

### B-cell Epitope Prediction

Prediction of B-cell linear epitopes of NSP2-CHIKV was performed with BepiPred Linear Epitope Prediction method using IEDB. The threshold was set to 0.35 to get accurate results with good specificity and sensitivity.

### T-cell Epitope Prediction

Peptides binding to MHC-I and MHC-II molecules were predicted using the IEDB tool. Peptides with high antigenicity binding to MHC-I were predicted for HLA-A\*01 and peptides binding to MHC-II were predicted for HLA-DRB\*01. HLA-A\*01 and HLA-DRB\*01 are genes which encode human protein to produce immunity.

## RESULTS

### Protein Alignment

Multiple protein sequence alignment reveals that NSP2 includes highly conserved residues while certain regions display variations. Conserved regions are shown with dots (Figure 1). Amino acids that are highly variable are denoted by respective amino acid residues. The alignment analysis clearly shows that there is high similarity among sequences of different strains from different countries. The residues having the greatest number of mutations are at positions 54, 374, 642, 643 and 793 as shown in Figure 1.

At position 54, Asparagine (N) is mutated to S (Serine) in 29 strains from different countries. Interestingly, they are both polar with no charge with a characteristic feature of H-bonding, suggesting no impact on functioning of NSP2.

At position 374, Tyrosine (Y) is mutated to Histidine (H) in 17 strains. Tyrosine is a polar amino acid with no charge while Histidine is a polar amino acid with positive charge, so this represents a shift from neutral to basic amino acid.

At position 642, there is a slight mutation at the Tyrosine residue with Cystine (C ). In 07 strains at position 642, Tyrosine is mutated

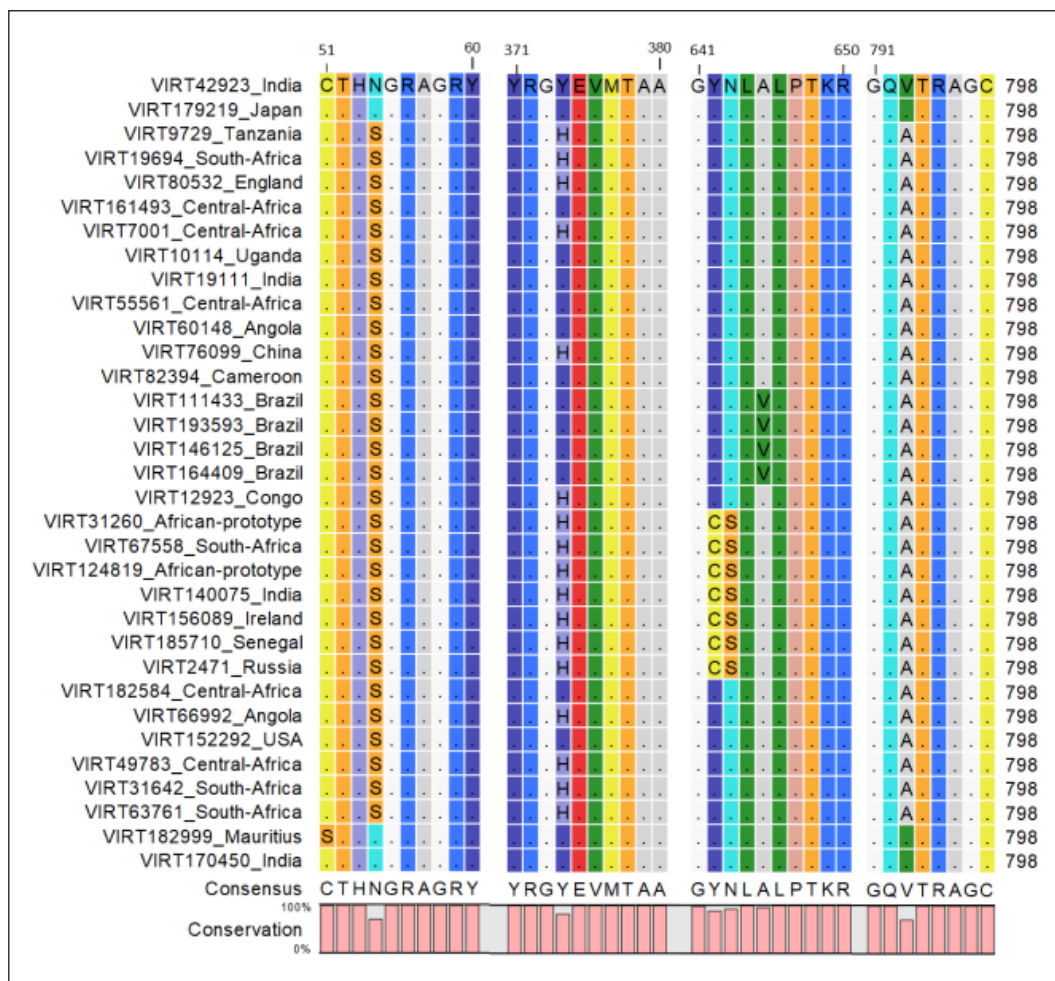


Figure 1. Amino acid sequence alignment of NSP2-CHIKV.

Analysis was performed with CLC main Workbench. Points represent conserved residues while mutated residues were only mentioned with their respective symbols.

to Cysteine (C). As both residues are polar, it is suggested that there is no impact on the function of NSP2. At position 643, 7 strains have mutations and these mutations are also found in strains from those countries which had the same mutations at position 642. N (Asparagine) is mutated to S (Serine), both are polar with no charge.

At position 793, Valine (V) is mutated to Alanine (A) in 29 different strains belonging to different countries. Interestingly, both are non-polar. Most interestingly, these 29 mutations at position 793 are found in strains from the same countries which have mutations at position 54, 374, 642 and 643. It shows there is some sort of linkage between strains of these different countries which include Tanzania, South Africa, England, Central Africa, Uganda, India, Angola, China, Cameroon, Brazil, Congo, Ireland, Senegal, USA and Russia. At the bottom we can see bars indicating percentage of residue conservation and variation, as shown in Figure 1.

### **Phylogenetic Tree**

A phylogenetic tree was constructed from 100 protein sequences of NSP2 CHIKV from all over the world using the UPGMA method (Figure 2). The root bifurcates in two interior branches, which share a common ancestor. First the interior branch further bifurcates into two branches. The first branch makes a short cluster or clade alone containing Reunion, India, Singapore and USA while the second sub-branch again divides into two branches. The second interior branch bifurcates into three branches which is further divided. This phylogenetic tree suggests different strains of NSP2 show many similarities, nevertheless, belonging to distinct countries. Contrarily, some strains are distantly related to each other within the same country. For example, strains from India are distantly related to each other and appear on distant clades; these differences might be due to traveling to other countries where CHIKV is epidemic.

### **Global Consensus Sequence**

In this study, multiple aligned protein sequences were subjected to analysis to develop a global consensus sequence (Figure 3).

### **B-Cell and T-Cell Epitope Prediction**

Epitopes are part of the antigen, to which B-cell or T-cell antibodies bind to produce immunity against the antigen.

#### **B-Cell Epitopes Prediction**

At least thirty-four short peptides for B-cell epitopes were predicted from global consensus sequences using the BepiPred epitope prediction method, as shown in Table 1. Based on scoring, the epitope VVDTTGSTKPDPGD at position 341-354 is considered to be most effective. The residues above a threshold of 0.35 have a higher probability of being part of the epitope and are represented as yellow, as shown in Figure 4. The higher the peak in a graph, the higher is the probability of being an effective epitope. If the threshold is set as high as 1, perfect specificity of the epitope for B-cells will be obtained but sensitivity will not be ideal, but at 0.35 default threshold, the correct specificity and sensitivity both will be obtained.

#### **T-Cell Epitope Prediction**

T-cell epitopes encode for cell surface proteins. MHC-I is found in all nucleated cells, which includes B-cells, dendritic cells, antigen presenting cells and macrophages. MHC-I molecules present antigens to cytotoxic T-cells having CD8<sup>+</sup> receptors. On the other hand, MHC-II molecules are usually found on professional antigen presenting cells which include mononuclear phagocytes, dendritic cells, thymic epithelial cells, B cells and some endothelial cells. It represents antigens to helper T-cells having CD4<sup>+</sup> receptors.



Figure 2. Phylogenetic tree of 100 homologous sequences of NSP2-CHIKV. The phylogenetic analysis was inferred using the UPGMA method.

### MHC-I T-cell Epitopes

Short peptide epitopes predicted for HLA-A are shown in Table 2. The lower the percentile rank, the higher is the affinity for the epitope. The best epitopes predicted for

HLAA\*01:01 are VTAIVSSLHY, SLSESATMVY, FSKPLVYY, QPTDHHVGEY at positions 317-326, 84-93, 535-544 and 15-24, respectively. They have the lowest percentile rank.





Figure 3. Global Consensus Sequence for NSP2-CHIKV.  
The global consensus sequence was achieved for NSP2 of CHIKV using CLC workbench.

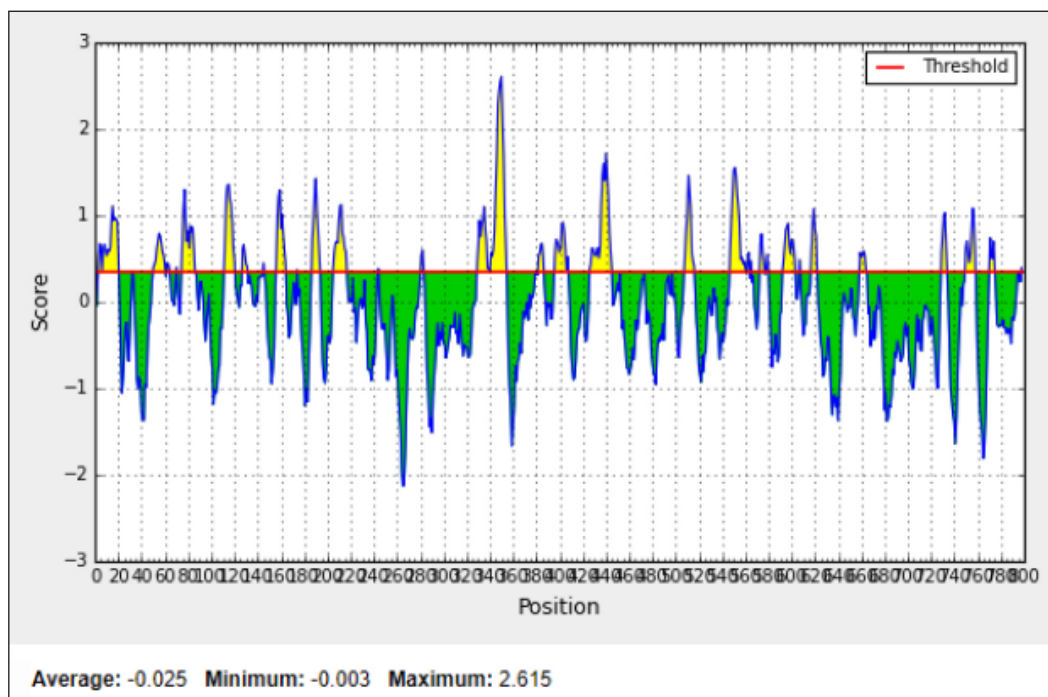


Figure 4. B-cell epitope prediction.  
Prediction of B-cell Linear Epitopes of NSP2-CHIKV was done using IEDB. Threshold was set to 0.35 to come up with high specificity and sensitivity.

Table 1. Prediction of short peptides for B cell epitopes

**Predicted peptides:**

No. ◆	Start ◆	End ◆	Peptide ◆	Length ◆
1	3	20	IETPRGAIKVTAQPTDHF	18
2	50	60	TCTHNGRAGRY	11
3	62	64	VEA	3
4	70	70	L	1
5	75	85	YAISPEDFQSL	11
6	111	120	PALNTDEESY	10
7	127	131	RTEHE	5
8	145	145	E	1
9	156	164	LTNPPYHEF	9
10	174	174	A	1
11	187	193	VPGSGKS	7
12	205	216	LVTSGKKENCQE	12
13	244	244	R	1
14	281	283	PKQ	3
15	329	339	KMRTTNEYNKP	11
16	341	354	VVDTTGSTKPDGPD	14
17	380	380	A	1
18	382	386	SQGLT	5
19	396	407	KVNENPLYASTS	12
20	426	445	LSGDPWIKTLQNPPKGNFKA	20
21	497	497	R	1
22	508	516	EDKAYSPEV	9
23	547	560	NHWDNRPGGKMFGF	14
24	562	563	PE	2
25	565	565	A	1
26	573	576	PFTK	4
27	578	580	KWIN	3
28	593	603	EDFNPTNIIP	11
29	607	607	R	1
30	617	621	RPVKG	5
31	659	664	VRGADY	6
32	729	733	YADRT	5
33	750	758	ALKPPCVTS	9
34	771	775	NGRRN	5

Table 2. T-cell class I MHC-specific predicted epitopes of the CHIKV polyprotein and their number of alleles and immunogenicity score

Allele	#	Start	End	Length	Peptide	Consensus Percentile Rank
HLA-A*01:01	1	317	326	10	VTAIVSSLHY	0.175
HLA-A*01:01	1	84	93	10	SLSESATMVY	0.39499999999999996
HLA-A*01:01	1	535	544	10	FSKPLVSVYY	0.515
HLA-A*01:01	1	15	24	10	QPTDHVVGEY	0.81
HLA-A*01:01	1	152	161	10	LVGDLTNPPY	1.04
HLA-A*01:01	1	51	60	10	CTHNGRAGRY	1.1
HLA-A*01:01	1	85	94	10	LSSESATMVYN	1.185
HLA-A*01:01	1	114	123	10	NTDEESYELV	1.21
HLA-A*01:01	1	466	475	10	MTFDTFQNKAK	1.415
HLA-A*01:01	1	16	25	10	PTDHVVGEYL	1.435
HLA-A*01:01	1	123	132	10	VRAERTEHEY	1.6
HLA-A*01:01	1	534	543	10	LFSKPLVSVY	1.9
HLA-A*01:01	1	757	766	10	TSNTEMFFLF	2.0999999999999996
HLA-A*01:01	1	720	729	10	GSLLIRAYGY	2.2
HLA-A*01:01	1	406	415	10	TSEHVNVLTT	2.6
HLA-A*01:01	1	517	526	10	ALNEICTRMY	2.65
HLA-A*01:01	1	686	695	10	IHTPFRIHHY	2.6500000000000004
HLA-A*01:01	1	156	165	10	LTNPPYHEFA	2.7
HLA-A*01:01	1	563	572	10	EAASILERKY	2.9
HLA-A*01:01	1	127	136	10	RTEHEYVYDV	3.3
HLA-A*01:01	1	377	386	10	MTAAASQGLT	3.8000000000000003
HLA-A*01:01	1	670	679	10	LGLPATLGRY	3.9
HLA-A*01:01	1	218	227	10	TTDVMRQRGL	3.95
HLA-A*01:01	1	756	765	10	VTSNTEMFFL	4.199999999999999
HLA-A*01:01	1	755	764	10	CVTSNTEMFF	4.35
HLA-A*01:01	1	89	98	10	ATMVYNREF	4.5

### MHC-II T-cell Epitopes

Short peptide epitopes predicted for HLA-A are shown in Table 3. The best epitope predicted for HLA-DRB\*01:01 is

VVGEYLVLSPTQTVLRS from 20-35 with the lowest percentile rank. The lower the percentile rank, the higher is the affinity for the epitope.



Table 3. T-cell class II MHC-specific predicted epitopes of the CHIKV polyprotein and their number of alleles and immunogenicity score

Allele	#	Start	End	Peptide	Consensus percentile rank
HLA-DRB1*01:01	1	20	34	VVGEYLVLSPTVLR	0.01
HLA-DRB1*01:01	1	21	35	VGEYLVLSPTVLR	0.01
HLA-DRB1*01:01	1	22	36	GEYLVLSPTVLR	0.03
HLA-DRB1*01:01	1	19	33	HVVGEYLVLSPTVL	0.05
HLA-DRB1*01:01	1	23	37	EYLVLSPTVLR	0.09
HLA-DRB1*01:01	1	371	385	YRGYEVMTAAASQGL	0.09
HLA-DRB1*01:01	1	24	38	YLVLSPTVLR	0.19
HLA-DRB1*01:01	1	18	32	DHVVGEYLVLSPTV	0.28
HLA-DRB1*01:01	1	372	386	RGYEVMTAAASQGLT	0.32
HLA-DRB1*01:01	1	370	384	DYRGYEVMTAAASQG	0.47
HLA-DRB1*01:01	1	369	383	IDYRGYEVMTAAASQ	0.79
HLA-DRB1*01:01	1	373	387	GYEVMTAAASQGLTR	0.88
HLA-DRB1*01:01	1	368	382	QIDYRGYEVMTAAAS	0.96
HLA-DRB1*01:01	1	374	388	YEVMTAAASQGLTRK	1.71
HLA-DRB1*01:01	1	117	131	EESYELVRAERTEHE	2.27
HLA-DRB1*01:01	1	710	724	GDRLRLKPGGSLLI	2.51
HLA-DRB1*01:01	1	711	725	DSLRLKPGGSLLIR	2.51
HLA-DRB1*01:01	1	116	130	DEESYELVRAERTEH	2.73
HLA-DRB1*01:01	1	256	270	ACHSGTLLALIALVR	3.24
HLA-DRB1*01:01	1	257	271	CHSGTLLALIALVRP	3.49
HLA-DRB1*01:01	1	258	272	HSGTLLALIALVRPR	3.49
HLA-DRB1*01:01	1	259	273	SGTLLALIALVRPRQ	3.49
HLA-DRB1*01:01	1	118	132	ESYELVRAERTEHEY	3.63
HLA-DRB1*01:01	1	260	274	GTLLALIALVRPRQK	3.72

## DISCUSSION

CHIKV is likely to be transmitted through *Aedes aegypti* and *Aedes albopictus* (Petersen & Powers, 2016). This infection takes place in aged individuals or patients with a compromised immune response. Currently, there is no FDA-approved vaccine against

CHIKV (Law *et al.*, 2019). Furthermore, there is no specific treatment against this disease and the generalized medication involves use of paracetamol, aspirin and anti-inflammatory drugs (Thiberville *et al.*, 2013). It is estimated that CHIKV evolved from ancestors that existed 500 years ago and the lineage is thought to start within

Africa. It is estimated that 70 to 90 years ago CHIKV was introduced into Asia from Africa (Volk *et al.*, 2010).

Previous studies demonstrated structural and non-structural proteins as targets to find the antigenic epitopes that could be effectively used as vaccine candidates (Islam *et al.*, 2012; Kori *et al.*, 2015; Nair *et al.*, 2015; Rashad *et al.*, 2013). Epitope prediction in a few studies is based on a single gene (NSP4, RNA-dependent RNA Polymerase) of CHIKV (Waheed *et al.*, 2017) or interaction of two CHIKV genes (NSP1 interaction with NSP2) (Kumar *et al.*, 2018). NSP2 and NSP3 of CHIKV exhibit activity of RNA interface (RNAi) suppression. RNAi is an antiviral mechanism pathway (Mathur *et al.*, 2016). The ability to recognise small peptide proteins of NSP2 CHIKV differs from NSP2 of other Alphaviruses (Saisawang *et al.*, 2015).

Humoral immunity is induced by B-cell epitope interaction with B-lymphocytes, followed by production of antibody-secreting plasma cells and memory cells (Shawan *et al.*, 2014). Vaccines have mostly been based on B-cell immunity (Parashar & Cherian, 2014; Powers, 2018) but *in-silico* approaches made it possible to induce T-cell immunity by predicting and designing vaccines against T-cell epitopes, which is supported because CD8<sup>+</sup> cells generate strong immunity against infected cells (Yasmin & Nabi, 2016). Epitope vaccines are easy to develop; manufacturing is cheap and has fewer side effects compared to conventional epitopes (Dar *et al.*, 2016). Epitope-based vaccines are thought to be promising vaccines for the next generation (Ayub, G *et al.*). MHC is a tightly linked set of genes that play an essential role in adaptive immunity and define the outcome of many host immune responses. Furthermore, the immunogenicity of the potential epitope is defined by its binding affinity to MHC molecule (Kumar Pandey *et al.*, 2018).

In the present study, NSP2-CHIKV has been analyzed using various *in-silico* tools. One hundred homologous sequences have been retrieved from databases and analyzed with the aim of determining the best possible

epitopes with high binding affinity and antigenicity.

We designed short peptides of amino acids/epitopes from highly conserved regions of NSP2-CHIKV. It is suggested that these epitopes could be incorporated into vaccine formulations to yield a better outcome in the human population.

This study predicts 34 B-cell epitopes and the promiscuous epitope is VVDTTGSTKPDPGD at position 341-354. Furthermore, 26 MHC-I epitopes are predicted to bind with HLA-A. The promiscuous epitopes include VTAIVSSLHY, SLSESATMVY, FSKPLVYY, and QPTDHHVGEY at positions 317-326, 84-93, 535-544 and 15-24 with the percentile ranks of 0.17, 0.39, 0.51 and 0.81, respectively. These have been predicted to bind with HLA-A\*01:01. In addition, this study predicts 24 MHC-II short peptide epitopes for HLA-DRB. The promiscuous epitope predicted to bind with HLA-DRB\*01:01 is VVGEYLVLPQTVLRS from 20-35 with lowest percentile rank of 0.01. These epitopes are suggested to be incorporated in the vaccine formulations to yield better status of human population.

CHIKV is evolving with time due to different reasons such as traveling, urbanization, lack of effective control measures, adaptation of virus and spread of new vectors and there should be an effective means to stop this disease from spreading. This study was conducted to analyze NSP2-CHIKV as it plays a crucial role in CHIKV replication so that epitopes can be analyzed for B-cell and T-cell affinity as effective antiviral drug design.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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