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A Bioinformatics Approach to Designing a Multi-Variant Vaccine Against *Entamoeba histolytica*

Adeoti O M[1, 2, 4*], Aderinto A O[1], Adesina D A[1,3], Adesanya V O[1] and Olaoye O J[1]

[*1]*The Oke- Ogun Polytechnic, Department of Science Laboratory Technology, Saki Oyo State, Nigeria*

[2]*Ladoke Akintola University of Technology, Department of Pure & Applied Biology, Ogbomoso, Nigeria*

[3]*Department of Botany and Microbiology, University of Ibadan, Nigeria*

[4]*Cellular Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria*

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ABSTRACT

Entamoeba histolytica is the parasite that is responsible for amoebiasis and remains one of the very important best three parasitic causes of mortality worldwide. With increased travel and emigration to developed countries; such infection is becoming more common in non-endemic areas. Although the bulk of people infected with *E. histolytica* remains asymptomatic, some presents with amoebic colitis and disseminated disease. As more is learned about its pathogenesis and thus the host's immune reaction, the potential for developing a vaccine holds promise. This narrative reported outline the present knowledge regarding *E. histolytica* insight within the development of a vaccine. During this study, immune informatics approach was used to design a completely unique epitope based oral vaccine against *E. histolytica*. This is due to the rise in resistance to antibiotics for the control, leading to an efficient vaccine as an alternative, which remains a challenge. Therefore, a rational, strategic, and efficient vaccine design against *E. histolytica* is important where the utilization of the foremost current bioinformatics tools like GEPTOP CELLO NCBI which some predictions results of which ADH2, PFP, PFKA, ITPK1, C4LUC7, HEXB, PPDK, O00851, FEN1, ADH1, PAP, CDK2, M7VYQ0, N9V912, M3S0V3 and M2R918 possessed a cytoplasmic location; LYS4and CPP2 were extracellular, whereas TBP1, and HEXA were predicted as outer-membrane proteins, none were inner-membrane and periplasmic. In this study, immune informatics approach was use to design a completely unique epitope based oral vaccine against E. histolytica. The vaccine designed fulfilled non-allergenicity, antigenicity, and appropriate solubility, relative molecular mass and isoelectric point.

Keywords: Immunoinformatics, Allergenicity, Entamoeba histolytica, Antigenicity, Multi-Variant -Vaccine

INTRODUCTION

Amoebiasis, or amoebic dysentery, could also be a term used to describe an infection caused by the protozoan *Entamoeba histolytica* [1]. Mostly infections were commonly known as asymptomatic, but invasive intestinal disease might occur manifested with several weeks of cramping, abdominal pain, watery or bloody diarrhea, and weight loss [1]. Disseminated, extra intestinal disease like liver abscess, pneumonia, purulent pericarditis, and also cerebral amoebiasis has been described [1]. Worldwide, it has been estimated that up to 50 million people are suffering from *E. histolytica*, primarily in developing countries, and it's liable for over 100,000 deaths a year [2]. Transmission generally occurs by the ingestion of infected water or food thanks to fecal excretion of cysts, and even fecal-oral transmission within household and through male homosexual activity [1,3,4]. Vaccines were currently being investigated in rodent and a non-human primate model appears promising. Using both native and recombinant kinds of the amoebic Gal/GalNAc lectin has been presented for protection against intestinal and liver infections [5]. Investigated a vaccine in baboon models, a natural host for *E. histolytica* [6]. Cholera toxin B subunit was co administered with Gal-lectin which

Corresponding author: Adeoti O M, The Oke- Ogun Polytechnic, Department of Science Laboratory Technology, Saki Oyo State, Nigeria, Tel: 08067285175; E-mail: txy23m@yahoo.com

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resulted in significant, moderate level of protection against E. *histolytica* reinjection. The vaccinated baboons displayed higher titers of intestinal anti-peptide IgA, intestinal anti-lectin IgA, and also serum anti-peptide IgG antibodies, followed up colonoscopy showed no sign of inflammatory colitis or amoebic invasion [6]. Targeting other components of *E. histolytica*, like serine-rich protein and thus the 29-kDa- reductase antigen, has been shown successful against ALA in rodent models [5]. More rigorous testing for the event of a successful vaccine against E. *histolytica* is warranted. Apart from identifying target immunogenic proteins, the mixture of doses, adjuvants, and boosts must be optimized [7]. The responses in animal models, non-human primates especially [6], suggest that it's going to be possible to develop a viable human vaccine, advancing to urge beyond the preclinical stage. Sadly, however, the induction of LTM has not yet been presented in animal models, which is the hallmark of a successful vaccine.

The technological advances within the fields of genomics, proteomics, human immunology, and structural biology have provided the molecular information for the invention and prediction by bioinformatics tools of novel antigens, epitopes, and style of vaccines against pathogenic bacteria, like meningococcus B [8-10]. Bioinformatics tools can predict sequences with binding affinity to MHC alleles and epitopes of T and B cells [11]. The main target on vaccine design and development has changed to the assembly of peptides composed of multiple epitopes (multi epitope vaccines), supported linear arrangements, as a totally unique alternative. Additionally, epitope-based vaccines have demonstrated various advantages, including safety, the chance to rationally engineer the epitopes for increased potency, breadth, and antigenicity, and therefore the possibility to focus large repertories of immune responses on conserved epitope sequences [12]. This study was an *in-silico* attempt aimed toward designing a completely unique oral vaccine against *E. histolytica* was *in silico* designed.

METHODS

Selection and Antigenic Evaluation of Protein

E. histolytica proteins were selected based on the following criteria: (1) reported antigenicity, (2) virulence, and (3) proteins related to the mechanisms of adhesion. The entire twenty protein sequences were retrieved from UniProt reference sequence database in FASTA format. For bacterial protein subcellular localization prediction, CELLO v2.5 was used [13]. The database of Gep Top was used to evaluate the essentiality of genes [14]. PATRIC3.5.16 database was used for study of the virulence role of proteins. Using BLASTp to screen proteins for detecting sequence homologs to *Homo sapiens*. Prediction of transmembrane (TM) helixes, TMHMM v2.0 was used. Compute pI/Mw tool was used to calculate the estimated isoelectric point and molecular weight of all amino acid sequences [15].

Phylogenetic Evolution

Phylogenetic tree of the retrieved sequence of the capsid protein of *E. histolytica* was created using Mega X software. 20 of the protein trees were constructed using maximum likelihood parameter in the software [16].

Conservation, Consensus Sequence and Alignment

Conservation of selected proteins was analyzed using E. histolytica strain 26695 as the reference. For proteins with high variability, a consensus sequence was generated using sequences of world E. histolytica representative strains (20 complete genome sequences). CLC Main Workbench v7.8 was performed for sequence alignment (QIAGEN Bioinformatics), Emboss Cons, and T-Coffee software.

T Cell Epitope Prediction

To identify MHC-I binding epitopes, NetMHC 4.0 server was used [17]. Fifty-one human leukocyte antigen (HLA) alleles (HLA-A, -B, -C, and -E) and six murine alleles (H-2) were evaluated. Base on the MCH-I binding epitopes predictions were calculated for nine-mers epitopes with a threshold for strong binders of 0.5% and a threshold for weak binders of 2%.

For MHC-II binding epitopes, NetMHCII 2.3 server; predictions which were obtained for 20HLA- DR alleles, 20 HLA-DQ, 9 HLA-DP, and 7 mouse H2 class II alleles using a threshold of -99.9, threshold for the strong binder of 5%, and threshold for the weak binder of 20%.

B Cell Epitope Prediction

Linear B cell epitopes of 20-mers were predicted utilizing ABCpred with a threshold of 0.7. The second was BCPred server which was applied with a specificity threshold of 75%. For BepiPred server, only amino acids with score >1.0 were considered for the downstream analysis [18].

Selection of Epitopes

They were selected based on the following criteria: (1) 20-mer epitopes, (2) epitopes matching on all algorithms, if possible, and (3) potential to bind with the maximum number of MHC-I and MHC-II alleles. Using Clustal Omega server for the selection of sequences that were aligned and overlapped.

Vaccine Design

Vaxign Server was used to get the vaccine design which shows the protein accession, gene symbol, localization probability, adhesion probability, transmembrane helices and the protein length [19].

Protein Prediction and Validation of Secondary and Tertiary Structures

The secondary structure of the multi-epitope antigen was predicted using PSIPREDv3.3 Protein Prediction and validation of secondary and tertiary structures [20]. The

three-dimensional (3D) structure modeling was performed using Swiss-Model server. Jmol was used for visualizing 3D structures of proteins. For refinement of 3D model structure, Galaxy Refine and Galaxy Loop were applied [21].

The best model was validated by the ProSA web and ERRAT. The residue-by-residue stereochemical qualities of models were validated by Ramachandran plot obtained from PROCHECK server [22]. The best-refined model was selected.

Antigenicity, Allergenicity, Solubility and Physicochemical Predictions of Vaccine

For antigenicity prediction, VaxiJen server was used for antigenicity, allergenicity, solubility, and physicochemical predictions of vaccine. AllerTOP v.2.0 and AlgPred servers were used for allergenicity evaluation. For solubility prediction, SOLpro server was used. Finally, ProtParam allowed the computation of various physical and chemical parameters [15].

RESULTS AND DISCUSSION

Protein Selection and Evaluation

It has been shown that the combination of a long list of antigens do increase the effectiveness of vaccines [23] evaluated a multi component vaccine in human volunteers; they observed that the vaccine was safe but highly immunogenic, inducing long-lasting humoral and cellular responses to the antigens. Twenty 20 proteins were selected for this vaccine candidate. These were further screened for the next step for further parameters, including subcellular localization, essentiality, virulence, non-human homology, TM helixes, and molecular weight. Our predictions showed that sixteen: ADH2, PFP, PFKA, ITPK1, C4LUC7, HEXB, PPDK, O00851, FEN1, ADH1, PAP, CDK2, M7VYQ0, N9V912, M3S0V3 and M2R918 possessed a cytoplasmic location; LYS4 and CPP2 were extracellular, whereas TBP1, and HEXA were predicted as outer-membrane proteins as shown in **Table 1**. Both surface and extracellular proteins are good targets to develop a vaccine aiming toward prevention of bacterial infections and diseases [24].

In the analysis of essential genes by using GepTop package, fifteen (15) out of the 20 selected proteins were predicted to have essential genes. ADH2, PFKA, ITPK1, C4LUC7, TBP1, HEXA CPP2, PPDK, O00851, FEN1, ADH1, LYS4, PAP, CDK2 and M2R918 were identified to have essential genes. Essential genes are those genes for an organism that are critical for its survival; essential genes are of particular importance because of their theoretical and practical applications such as studying the robustness of a biological system, defining a minimal genome/organism and identifying effective therapeutic targets in pathogens [14]. Homology analysis of the 20 prioritized proteins using BLASTp revealed <60% identity, which was significant and important to declare the sequences as nonhuman homologs; A good vaccine targets should not be human homologues so as to avoid autoimmunity. The prediction of the topology of proteins by TMHMM showed that none of them have helix. Finally, molecular weights calculated by Compute pI/Mw tool of 4 proteins resulted to weigh <60,161.14 kDa respectively, also 2 protein resulted to weight <110000 (C4LUC7andO00851) whereasADH2, PFKA, ITPK1, TBP1, HEXA CPP2, PPDK, FEN1, ADH1, LYS4, PAP and CDK2 have molecular weights of 95658.72, 60161.14, 47669.98,36480.07,25966.95,63934.85,64371.51,34688.07, 98060.39,42577.12,38567.57,22379.07,59810.12 and 33845.11kDa, respectively **(Table1)**.

Good vaccine candidates are considered as those that do not have similarity with human proteins in order to avoid or prevent the generation of a potential autoimmune response; they must also lack TM regions, to facilitate their expression. Other features of a good vaccine potential are that it should possess good antigenic properties, which are vital for disease pathogenesis and for protection against the antigen [25]. Some authors have used these approaches in the selection of candidate proteins for the*in silico* design of *E. coli* vaccines [26].

T and B Cell Epitopes

The prediction analysis by different bioinformatics servers for T and B cells (using MHC-I/-II alleles for human and mouse BALB/c) allowed the selection of 20 epitopes based on their score, number of alleles, and agreement between the servers used. Epitopes segment obtained were: $ADH2_{23-42}$, PFP_{28-47}, $PFKA_{11-30}$, $ITPK1_{191-210}$, $C4LUC7_{748-767}$, $TBP1_{25-44}$, $HEXA_{453-472}$, $HEXB_{451-470}$, $CPP2_{59-78}$, $PPDK_{92-111}$, $O00851_{915-934}$, $FEN1_{107-126}$, $ADH1_{87-106}$, $LYS4_{152-171}$, PAP_{87-106}, $CDK2_{123-142}$, $M7VYQ0_{28-47}$, $N9V912_{132-151}$, $M3S0V3_{330-348}$ and $M2R918_{447-466}$ **(Table 2 and Figure 1).**

Protein Structure Prediction

The vaccine is composed of 400 amino acids, and prediction of secondary structure showed that it contains 67% a helixes, 15% ∝ sheets, and 18% others (random coil and □-turn), as shown in **Figure 3**.

Five 3D models of protein vaccine were generated among which the model with the highest c-score = 5 was selected for further refinement; the c-score range is typically from 5-6, the higher the value, the higher the confidence.

The quality and potential errors in the best model were analyzed. The initial input model z-score was-9.94, which falls within those commonly observed in similar size-native proteins **(Figure 4A)**. ProSA-web indicated that the preliminary model requires refinement processes. Hence, the raw model was subjected to loop refinement and energy minimization using galaxy refine. After all refinement procedures, ERRAT factor was improved from 96.8 to 97.9. The z-score of the final model reached a value of -10.11

Table 1. The subcellular localization, gene essentiality, virulence, human homology, transmembrane helix, isoelectric point and molecular weight predictions of *Entamoeba histolytica* selected proteins.

Protein	Accession no	Subcellular localization	Gene essentiality	Human homology	TM helix	pI/MW (kDa)
ADH2	sp|Q24803|	C-2.256	ES	NH	0	7.26 /95658.72
PFP	sp|C4LZC2|	C-4.292	N-ES	NH	0	6.34 / 60161.14
PFKA	sp|Q27651|	C-3.692	ES	NH	0	6.89 / 47669.98
ITPK1	sp|Q9XYQ1|	C-4.219	ES	H	0	5.95 / 36480.07
C4LUC7	tr|C4LUC7|	C-2.822	ES	NH	0	6.28 /119422.31
TBP1	sp|P52653|	OM-1.688	ES	NH	0	9.27 / 25966.95
HEXA	sp|P49009|	OM-3.020	ES	NH	0	6.48 / 63934.85
HEXB	sp|Q86M34|	C-1.404	N-ES	NH	0	5.56 /64371.51
CPP2	sp|Q01958|	E-2.947	ES	NH	0	6.88 / 34688.07
PPDK	sp|P37213|	C-3.818	ES	NH	0	5.96 /98060.39
O00851	tr|O00851|	C-2.994	ES	NH	0	5.87 /119552.21
FEN1	sp|C4M6G8|	C-3.544	ES	NH	0	8.90 / 42577.12
ADH1	sp|P35630|	C-3.963	ES	NH	0	6.10 / 38567.57
LYS4	sp|Q27650|	E-3.606	ES	NH	0	9.29 / 22379.07
PAP	sp|Q51D88|	C-2.113	ES	NH	0	5.39 / 59810.12
CDK2	sp|Q04770|	C-4.395	ES	NH	0	7.62 / 33845.11
M7VYQ0	tr|M7VYQ0|	C-4.292	N-ES	NH	0	6.34 / 60161.14
N9V912	tr|N9V912|	C-4.292	N-ES	NH	0	6.34 / 60161.14
M3S0V3	tr|M3S0V3|	C-4292	N-ES	NH	0	6.34 / 60161.14
M2R918	tr|M2R918|	C-4292	ES	NH	0	6.34 / 60161.14

aa: amino acid; C: cytoplasmic; E: essential; ES: extracellular; N-ES: nonessential; N-H: non-homology; N-V: nonvirulent; OM: Outer membrane; P: periplasmic; TM: transmembrane; V: virulent

Table 2. Potential Antigenic Epitopes Predicted by Different Servers.

				B cell Epitope			T Cell Epitope	
							NetMHC	
Order	**Protein**	**Position**	**Sequence**	**BCPRED**	**BepiPred**	**ABCpred**	**MHC 1**	**MHC II**
1	ADH2	23-42	ALKEYLKPEYTQEKIDYIVK	0.970	0.888	0.660	3H 1M	4H 2M
2	PFP	28-47	TIEEGAKTQSAVNQKELSEL	0.879	0.939	0.870	49H 2M	33H 3M
3	PFKA	11-30	IPKNPDAPLPSLKIEEVGEC	0.528	0.999	0.800	21H 1M	3H 3M
4	ITPK1	191-210	TRTSLPNVHRCGIKSVDFNN	0.844	0.856	0.870	21H 1M	7H 11M
5	C4LUC7	748-767	KGKKERRRESVDYTNNKWTA	0.970	1.000	0.890	10H 1M	9H 1M
6	TBP1	25-44	QLGNICHADYMSTSTESQER	0.727	0.911	0.830	30H 2M	81H 14M
7	HEXA	453-472	VNTHSMWVWTNRDMYDNDPV	0.723	0.935	0.960	33H 1M	50H 7M
8	HEXB	451-470	YVADSCTNPNHMWVWTNRDM	0.548	0.869	0.940	24H 1M	12H 6M
9	CPP2	59-78	VDGPFAAMTNEEYRTLLKSK	0.903	0.938	0.900	31H 1M	56H 10M
10	PPDK	92-111	SGAAMSMPGMMDTILNLGLN	0.675	0.863	0.890	6H 0M	9H 2M
11	O00851	915-934	FKKDDGPKTLPVEEQKSFSK	0.552	0.990	0.780	13H 0M	2H 10M
12	FEN1	107-126	KAQEQLDKALEEGDKEQAKK	0.751	0.792	0.880	12H 2M	9H 6M
13	ADH1	87-106	TPDWGEEESQRGYPMHSGGM	0.773	0.997	0.810	22H 0M	39H 8M
14	LYS4	152-171	PLWYPHYDNSPSFSDFSSFG	0.964	0.999	0.740	33H 1M	63H 15M
15	PAP	87-106	KRSITKSQEIKETSSQVPSS	0.925	0.996	0.830	34H 3M	60H 17M
16	CDK2	123-142	HRDMKPQNILINKNGTIKLG	0.756	0.738	0.940	24H 2M	15H 0M
17	M7VYQ0	28-47	TIEEGAKTQSAVNQKELSEL	0.782	0.939	0.870	37H 2M	28H 7M
18	N9V912	132-151	RNTGGFDLVGSGRTKIETEE	0.923	0.992	0.710	3H 1M	1H 0M
19	M3S0V3	330-348	LAHKKEEYSKITEFSAQKAF	0.879	0.824	0.850	13H 0M	18H 10M
20	M2R918	447-466	ALKKTGQICCISGLQKPAEE	0.619	0.832	0.640	15 0M	24H 12M

H: Human; M: Murine

(Figure 4B). The starting model was given **(Figure 5)**.

To validate the 3D models, Ramachandran plot analysis was performed before and after refinement processes. The Ramachandran plots of the unrefined model indicated that 88.1%of residues were located in most-favored regions, 11.3% in the additional allowed region, 0.4% in generously allowed regions, and 0.2% in disallowed regions of the plot **(Figure 6A)**.

The refined model showed that 94.5% of residues were located in most-favored regions, 5.3% in additional allowed regions, 0.0%ingenerously allowed regions, and only 0.2%in disallowed regions **(Figure 6B)**.

Antigenicity, Allergenicity, Solubility and Physicochemical Parameters of the Vaccine

An antigenicity score of 0.33722625 was obtained. The allergenicity prediction showed that the vaccine is allergenic. The molecular weight and theoretical pI of protein were 45189.22 kDa and 6.02, respectively. The final model was given **(Figure 7)**.

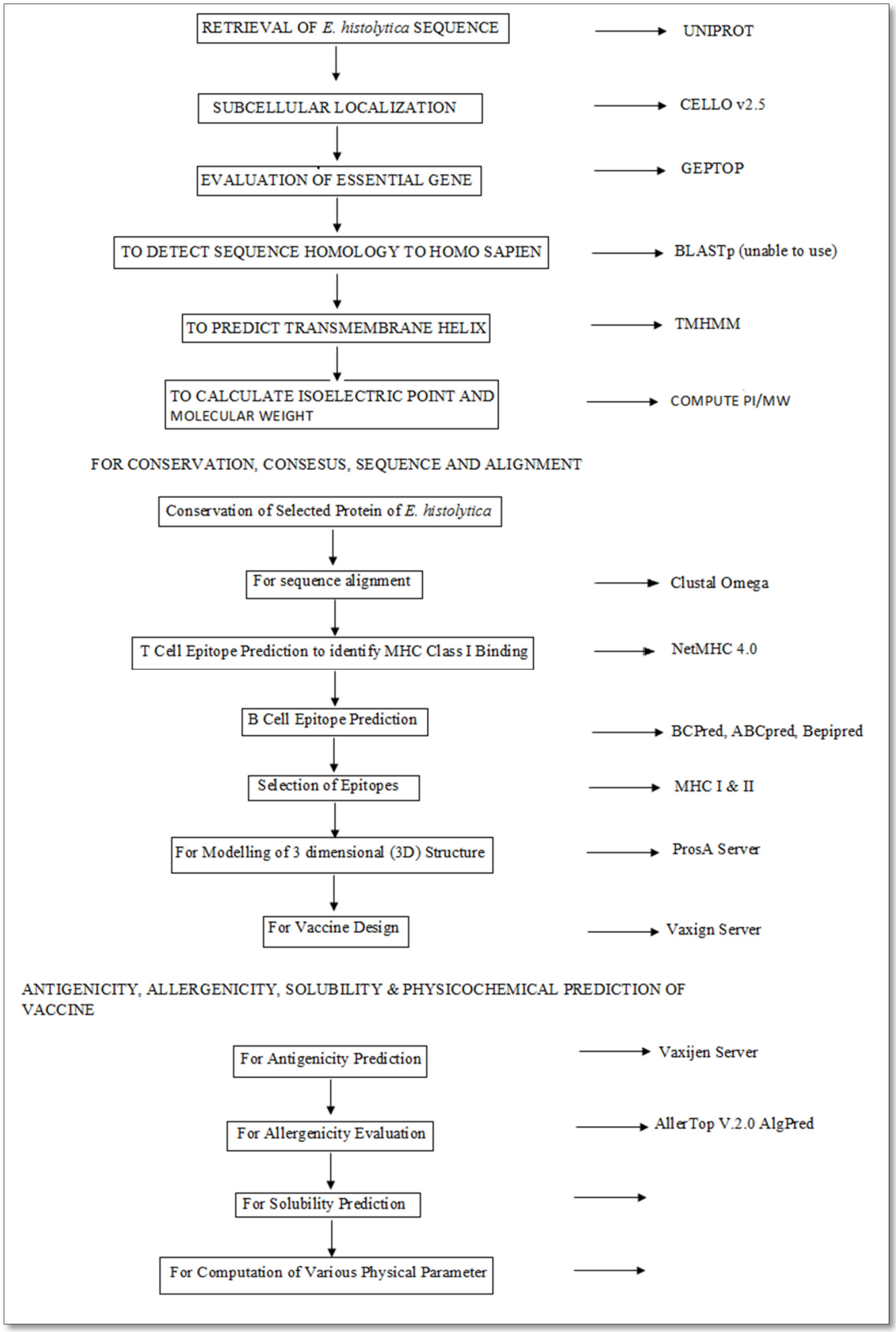

Figure 1. Flowchart for the Design of Novel Epitope for *E. histolytica.*

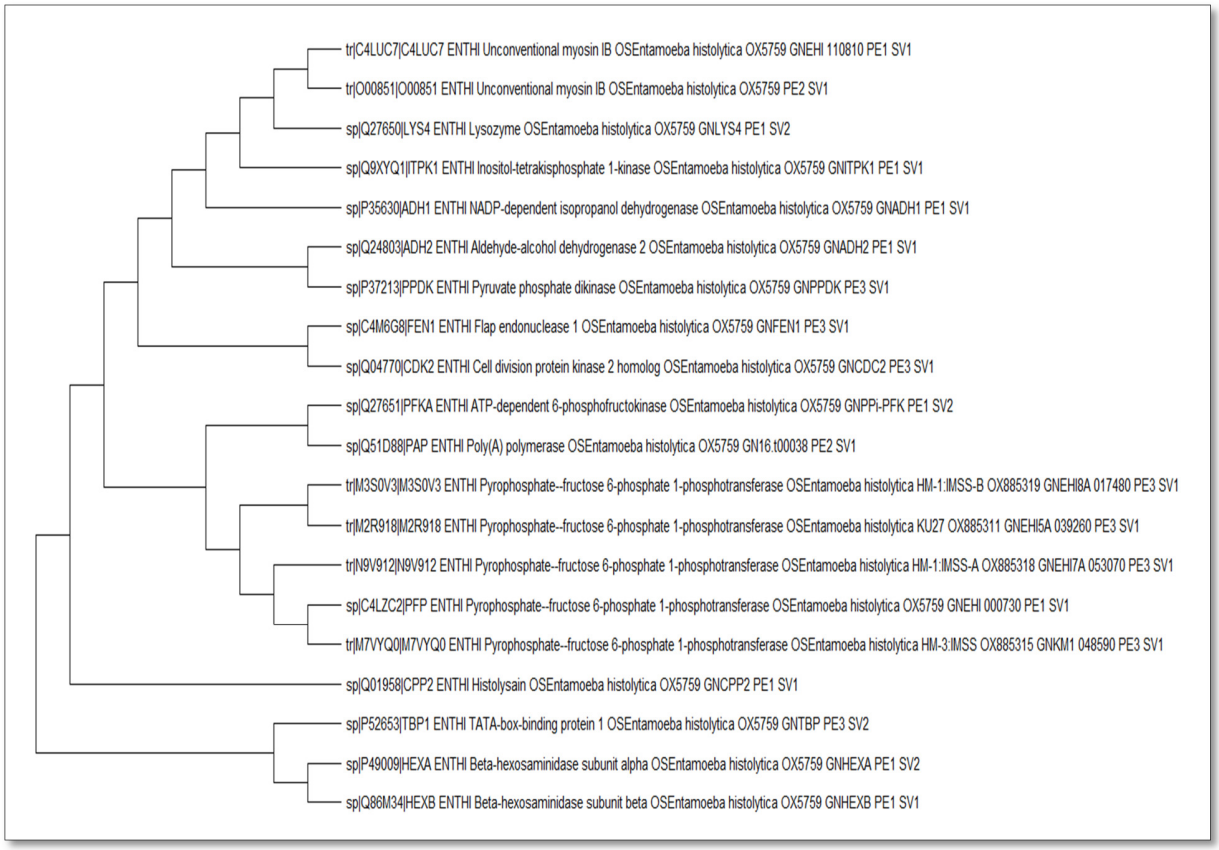

Figure 2. Phylogenetic tree for the selected capsid protein of *E. histolytica* created by Mega X [18].

CONCLUSION

In this study, we designed a novel multi epitopes oral vaccine against *E. histolytica* based on bioinformatics analysis to produce a huge and robust cellular and humoral immune response for *E. histolytica* prevention. This novel oral vaccine design could be a good vaccine candidate against *E. histolytica.* However, to make the therapeutic and prophylactic effect of our oral vaccine design valid, *in vitro* and *in vivo* immunological studies are required.

Figure 3. The secondary structure prediction of vaccine by PSIPRED. The protein vaccine consists of 15% a helix (H, cylinder), 18% b strand (E, arrow), and 67% coil (C, line) secondary structural elements. The bar chart represents the percentage of confidence.

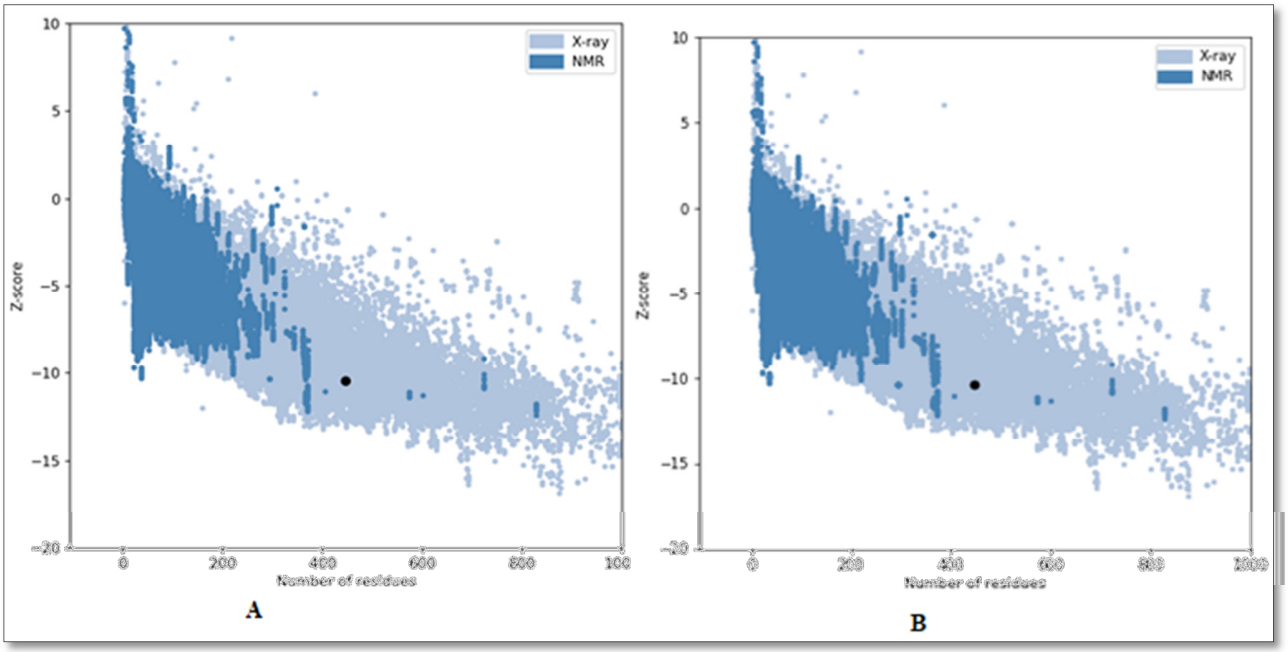

Figure 4. The z-score plot of unrefined and refined 3D structure of vaccine by ProSA-web. (a) The z-score of the starting model is -9.94; (b) The z-score of models after refinement steps is -10.11.

The z-scores indicates overall model quality and is depicted as a black spot. The z-scores of all experimentally determined protein chains in current protein data bank (PDB) from NMR spectroscopy (Charcoal) and X-ray crystallography (silver). 3D, three dimensional.

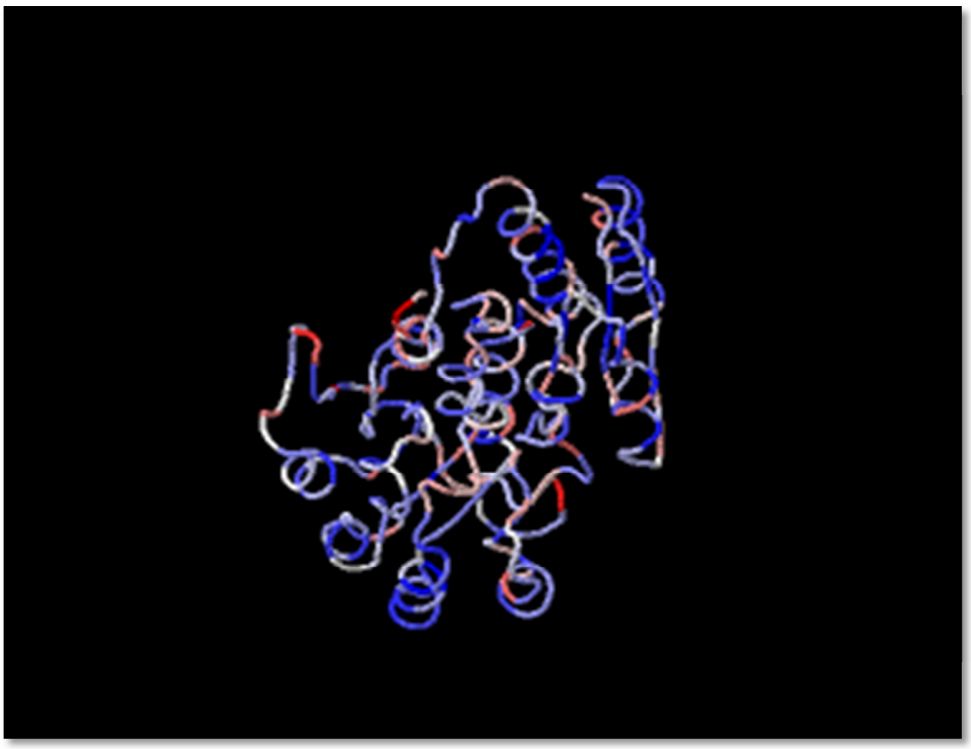

Figure 5. Unrefined 3D protein Structure of the vaccine by ProSA-web server.

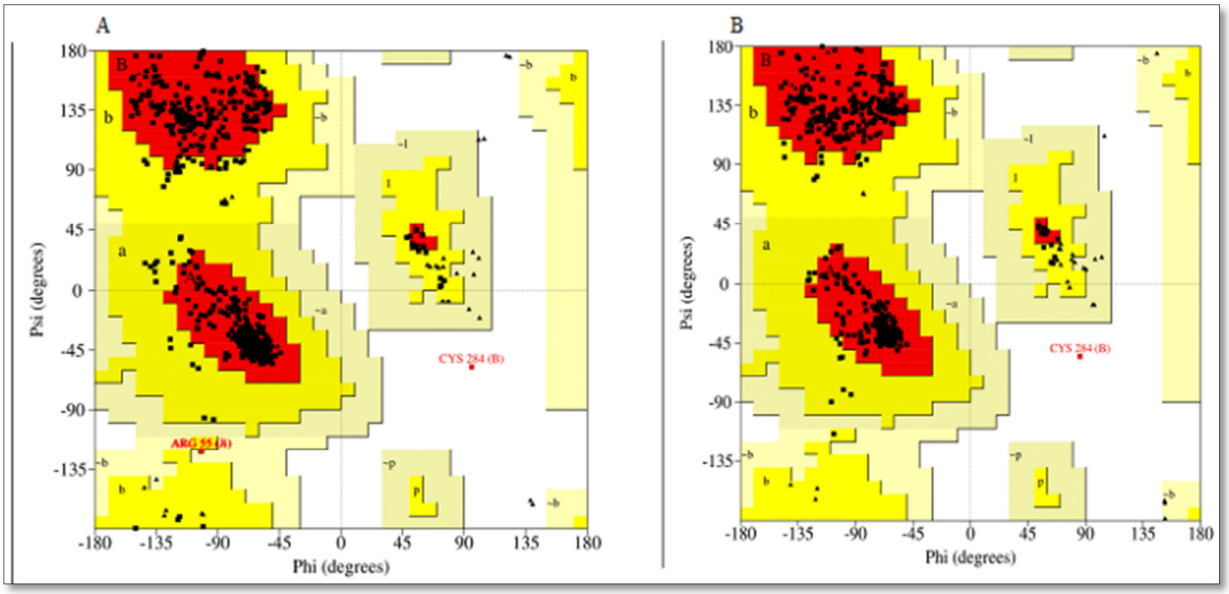

Figure 6. Validation of vaccine 3D model using Ramachandran plots of (a) the unrefined model and (b) the refined model. The most favored (A, B, and L) and additional allowed (a, b, l and p) regions were demonstrated with charcoal and silver-gray colors respectively. The generously allowed regions (-a, -b, -l and -p) are indicated in silver, and the disallowed regions are in white color. Glycine residues are shown in black triangles and other residues of protein are shown in black squares.

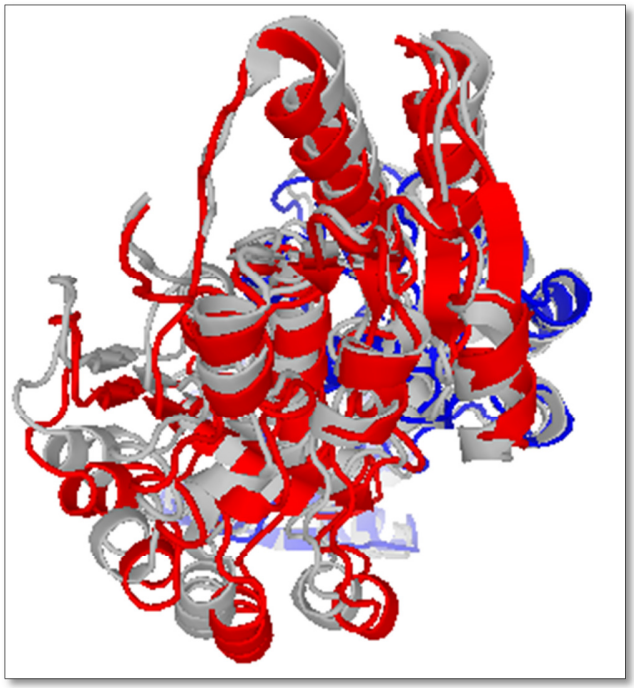

Figure 7. The recombinant protein vaccine solubility upon over expression in *Entamoeba histolytica* was 0.636769 insoluble with probability. The vaccine was found as unstable within instability index of 54.43. GRAVY and aliphatic index were assessed as -0.912 and 58.77 respectively.

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