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Research Article

In-silico Vaccine Candidate Peptides Against Urinary Tract Infection in Humans Using *Uropathogenic Escherichia Coli-Uti89* Strain

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Abstract

The rising antibiotic resistance of UPEC strains highlights the need for alternate preventive measures for urinary tract infections (UTIs) brought on by *Uropathogenic Escherichia coli (UPEC)* strain Uti89. This in silico study (2022-25) used a subtractive genomics-based reverse vaccinology approach; data extraction, protein analysis, epitope prediction, docking analysis, protein-ligand complexes, molecular dynamics, Human immune simulation, fragment Inserted in vector (pET-28a (+) were all steps in the study's multi-step methodology to find a potential vaccine candidate against UTIs. 3,840 non-homologous proteins unique to *UPEC-UTI89* were found by extracting data from databases. The pathogen's essential genes were identified, and predict on the bases of subcellular localization assisted in choosing among membrane-related proteins that would make good vaccine targets. Seventy-one (71) proteins were identified through antigenic analysis as potential vaccine candidates. 7 outer membrane proteins with potential vaccine properties were found through screening and shortlisted for vaccine construction. Investigation of the metabolic pathways in UPEC-UTI89 revealed both typical and unusual pathways. Several tools were used to predict epitopes for B-cell and T-cell immune responses. MHC-I and MHC-II cluster analyses were carried out to confirm the prediction of potential epitopes. The resulting epitopes demonstrated potential for eliciting a powerful immune reaction against UTIs brought on by *UPEC* UTI89. By identifying potential vaccine targets and epitopes using subtractive genomics and reverse vaccination approach, this in silico study aids ongoing efforts to develop an effective UTI vaccine. The resulting epitopes demonstrated potential for eliciting a powerful immune reaction against UTIs brought on by *UPEC* UTI89.

Keywords

Subtractive Genomic Analysis, Reverse Vaccinology Approach, Epitope Prediction, Vaccine Construct, UPEC-UTI89

1. Introduction

Urinary tract infections, or UTIs, are among the most common bacterial infections around the world, with annual estimates of 404.61 million cases, 236,790 fatalities, and 520,200 DALYs [1, 22]. The burden of UTIs is skewed towards women, who experience a lifetime risk of 60% compared to 13% for men [2]. About 11% of girls and 7% of boys have had a UTI by age 16. The majority of young sexually active females experience UTIs (0.5-0.7 cases per person per year) [3],

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whereas in young males, it is infrequent (0.01 cases per person per year). The most common type is bladder infections, and the responsible microbes for community-acquired cases are enteric uropathogenic E. coli, accounting for 80-85% [5].

Historically, UTIs were reportedly described as early as 1550 BC in the Ebers papyrus. Treatment methods progressed from herbal remedies in the distant past to using antibiotics in the 20th century. Any portion of the urinary system the urethra, ureters, kidneys, or bladder can be affected by a UTI, and it can cause serious inflammation of the kidney, pyelonephritis. Most UTIs are caused by E. coli; healthcare-associated UTIs [11] can be due to other pathogens like Pseudomonas and Klebsiella.

The bacterium E. coli was discovered by Theodor Escherich in 1885 and is one of the most common pathogens causing UTIs. Several serotypes of this bacterium exist, some strains of which are extremely virulent. Uropathogenic E. coli (UPEC), as it contains virulence factors, including adhesins and toxins, which contribute to the pathogenic potential, is capable of colonizing the urinary tract via its fimbriae. UPEC strains, which may result from transmigration from the intestines into the urinary tract, might cause infection with their complex interaction with host cells [4, 14].

For long, antibiotic resistance has been one of the toughest challenges in UTI treatment. About 92% of bacteria causing UTIs are resistant to more than one commonly prescribed antibiotic (control). The resistance makes their treatments complicated and increases healthcare costs because of the misuse of antibiotics [16]. Therefore, UTI vaccines are badly needed to combat this outbreak. The current approach to vaccine development has been followed based on some virulence factors like fimbriae and toxins; some vaccines exist in some countries, like Uro-Vaxom and Solco-Urovac, although they are not available worldwide. Since a major challenge continues to be the development of a vaccine against UTIs, new approaches such as reverse vaccinology and subtractive genomics are being used in selection for a new vaccine. These technologies depend on genomic information to identify required proteins and epitopes for targeted immune response approaches [9, 12].

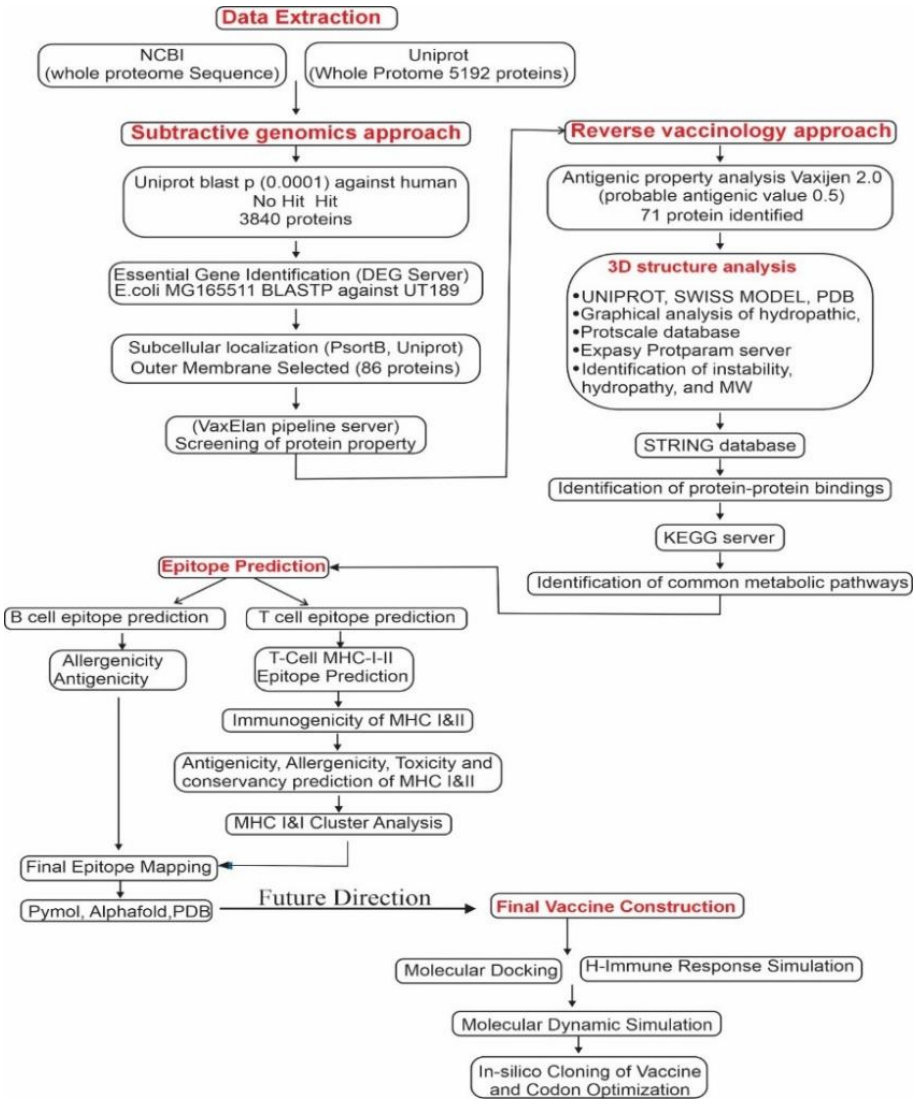


Figure 1. *flowchart for subtractive genomics and reverse vaccinology approach.*

Our study aims to predict possible vaccine candidates by computational methods through subtractive genomics to identify essential non-homologous proteins and reverse vaccinology for the prediction of B and T cell epitopes. This approach highlights the need for developing targeted and effective vaccines against UPEC strains.

2. Materials and Methods

The UPEC UTI89 strain was used in the current investigation to apply a subtractive genomics-based reverse vaccinology approach for the purpose of identifying vaccine against urinary tract infection. Several bioinformatics tools were used in the study to identify and prioritize the therapeutic vaccine using the UPEC UTI89 strain. The study protocol followed in this research is shown in flow chart Figure 1. They describe the procedure followed in the remaining study, respectively.

2.1. Data Extraction

The databases National Center of Biological Information (NCBI) (*https://www.ncbi.nlm.nih.gov/*) and the Universal Protein Resource (UniProt) (*https://www.uniprot.org/*) are used for the retrieval of the proteome sequence of the UPEC UTI89 strain of E. coli. A total of 5192 proteins of UPEC UTI89 strain were obtained from UniProt. The FASTA format sequence of the proteins was acquired from UniProt and subjected to further analysis.

2.2. Subtractive Genomic Approach

This approach plays an important role in potential target identification that is unique and essential for the pathogen but does not alter the host immune system. The entire proteome of E. coli. UPEC UTI89 is analyzed using the subtractive genomic techniques. This analysis is used to validate the specificity of the protein to reduce the chances of errors in the development of the novel vaccine.

2.2.1. Non-Homologous Protein Identification

The protein sequence retrieved from UniProt was subjected to UniProt, BLASTp with a p-value (0.0001) against H. sapiens, and it resulted in two forms: HIT or NO-HIT. HIT results in protein sequences homologous to human, and NO-HIT results in protein sequences non-homologous to H. sapiens. The proteins resulted in no hits being selected for further analysis.

2.2.2. Essential Gene Identification

For the identification of essential genes. DEG (Database of Essential Genes) (*http://www.essentialgene.org/*) server is used, for this purpose, we BLASTp E. coli MG1655II (the reason behind this selection of MG1655II instead of UPEC UTI89 is that the essential genes of the UPEC UTI89 strain were not completely identified yet.) against UPEC UTI89.

2.2.3. Subcellular Localization

The cellular location study is an essential step to describe sustainable medicine or vaccine targets. The subcellular localization of short-listed proteins was determined through PsortB (*https://www.psort.org/psortb/*) and the UniProt database. The proteins were selected in terms of membrane-related proteins [cell inner membrane proteins, outer membrane proteins, cell membrane proteins, periplasmic proteins, pilus proteins, bacterial flagellum basal body proteins, and other GO (gene ontology) annotation-based localization proteins]. Cytoplasmic proteins were not selected. As outer membrane proteins are better vaccine candidates. Therefore, outer membrane proteins were shortlisted for the screening of the vaccine candidate's prioritization. Screening of Protein Properties For further validation of protein properties, the VaxElan Pipeline server (*https://vac.kamalrawal.in/vaxelan/v2*) is used. Such as adhesion property, non-bacterial pathogens, antigen probability, non-allergen, MHC, IEDB class-I bonding prediction, class-I MHC binding, TM helices, virulence factor, CTL epitope prediction, non-homology, subcellular localization, cleavage sites, stability, molecular weight, and Pi and Si values.

2.3. Reverse Vaccinology Approach

The study of conventional vaccinology has evolved into a reverse vaccinology approach as a consequence of recent evolution in the field of proteomics and immunological research in the field of genetics and genomes. Reverse vaccinology was applied to the shortlisted probable outer membrane proteins chosen for vaccine candidate.

2.3.1. Antigenic Property Analysis of Essential Outer Membrane Proteins

The ability to enhance an immunological response when it is encountered by the human body in terms of antigenicity. Though proteins found inside of cells may serve as vaccine targets, it has been found through extensive research that vaccine candidates would be better served by membrane proteins that give immunity. The total of 86 outer membrane proteins was analyzed through Vaxijen v2.0 (*https://www.ddg-pharmfac.net/vaxijen/*) with a probable antigenic value of 0.5. Out of the total outer membrane proteins, 71 proteins of UPEC UTI89 resulted; they possessed an average ratio greater than the required value. 0.5.

2.3.2. Structure Analysis of Shortlisted Proteins

Furthermore, bioinformatics tools PDB (*https://www.rcsb.org/*), UniProt (*https://www.uniprot.org/*), and SWISS-MODEL (*https://swissmodel.expasy.org/*) [22] are used for the structure analysis of proteins. These servers' result in a 3D structure (3D) of the proteins by providing information like b

eta sheets, alpha helices, coils, and bindings in each and e very single portion of the protein structure statistics.

2.3.3. Graphical Analysis of Hydropathy, Kyte & Doolittle

It is important to visualize the hydropathic property of protein to check their structural complex-ability and inner membrane region. The graphical analysis provides information on the contraction of protein structure. Protscale database (*https://web.expasy.org/protscale/*) is used for the graphical analysis of hydropathic, Kyte, and Doolittle proteins.

2.3.4. Instability, Hydropathy, and Molecular Weight Demonstration

For the identification of instability, hydropathy, and molecular weight of proteins The Expasy Protparam server (*https://web.expasy.org/cgi-bin/protparam/protparam*) [19] is used. Protparam describes the statistical conditions where protein becomes unstable. A high ratio of hydropathy and molecular weight increases toughness, complex-ability, and chemical stress.

2.3.5. Identification of Protein-Protein Bindings

To identify how a protein interacts with other proteins 07 shortlisted high antigenic value protein attractions were verified through the STRING database (*https://string-db.org/*). The PPIs value helps to understand the physical as well as the functional interaction between these proteins [19]. To analyze the stability and instability of the protein positions, they are described by colors shown. Partially or covalently attaching the proteins with each other using the STRING database [7].

2.3.6. Protein Involves in Metabolism

The potential vaccine candidates and non-homologous protein pathogenic pathways retrieved on the above analysis were determined by using the Kyoto Encyclopedia of Genomes and Genes (KEGG) (*https://www.genome.jp/kegg/pathway.html*) [20]. KEGG server performs a comparison among the host (H. sapiens) and pathogens. They were used for the identification of common and unique metabolic pathways. Unique metabolic pathways are essential for the pathogen (E. coli) and can be retrieved from UniProt, KEGG, and NCBI.

2.3.7. Epitope Prediction

The technique of locating a particular antigen (often a protein) that can be recognized by antibodies or T cell receptors is known as epitope prediction. It is significant to emphasize that the accuracy and dependability of epitope prediction methods can vary and that the results are frequently enhanced by combining experimental and computational methods [13].

2.4. Prediction of B Cell Epitopes

Predicting epitopes of B cell receptors is the process of determining which affected parts of the human body are predicted to be recognized by B cells and to induce the antibody activity. B cell epitopes permit B lymphocytes to recognize antibody effects against an antigen that are found in any particular location in order to induce humoral immune reactions. Epitopes of B cells were identified using the IEDB (*https://www.iedb.org/*) and ABCpred (*http://crdd.osdd.net/raghava/abcpred/*) servers with a threshold score >0.8 selected. Additionally, to validate B cell epitope results, the tools Chou and Fasman beta-turn study, Karplus and Schulz flexibility study, Imini accessibility surface study, Kolasker & Tongaonkar antigenicity, and Parker Hydrophilicity prediction [17] are applied for the validation of epitopes of B cells.

2.4.1. Allergenicity and Antigenicity Prediction

The total B-cell epitopes were analyzed through Vaxijen v2.0 (*https://www.ddg-pharmfac.net/vaxijen/*) with a probable antigenic value of 0.5. Epitopes that possess a probable antigenic ratio greater than 0.5 were used for further analysis. It is important to verify the allergenicity of epitopes to enhance the efficiency of the vaccine candidate. Allergenicity of epitopes was analyzed through AllerTopV.2.0 (*https://www.ddg-pharmfac.net/AllerTOP/*), AllergenPRO, and AllergenFP.V.1.0 (*https://ddg-pharmfac.net/AllergenFP/*). The length of peptides was set to be at 15-18 mers. MHC-II epitopes were also screened on the basis of percentile rank <0.2 and IC50 value. The low score of IC50 and percentile ranks provides higher affinities of peptides. These selected epitopes were used for the remaining study.

2.4.2. Allergenicity, Conservancy, Toxicity, and Antigenicity Prediction of MHC-II

The selected MHC-II peptides on the basis of the IC 50 value and percentile rank were further determined on the basis of toxicity, conservancy, antigenicity, and allergenic properties. To identify the antigen value of the epitopes, use the Vaxijen v2.0 tool (*https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html*) with a probable threshold value. 0.5. The ToxinPred database (*http://crdd.osdd.net/raghava/toxinpred/*) is used to determine the relative toxicity level of epitopes. Where the IEDB peptide conservancy database (*http://tools.iedb.org/conservancy/*) was utilized to predict the conservancy of peptides. And the bioinformatic tools AllerTop V.2.0 AllergenFP.V1.O (*https://ddg-pharmfac.net/AllergenFP/*) and AllergenPRO are used for the prediction of the allergenicity of selected peptides.

2.4.3. MHC I-II Cluster Analysis

The MHC-cluster server (*https://services.healthtech.dtu.dk/services/MHCcluster-2.0/*). was utilized to check the potential and accurate peptide prediction of MHC class I-II. The heat map and phylogenetic tree generated by M

HC-cluster indicate how HLA alleles and peptides intera ct with each other and serve various functions [18].

2.4.4. Epitope Mapping

To analyze the selected epitopes in the protein structure, peptide mapping analyses are performed. Foe epitope mapping PDB File, Swissport Model, and PYMOL databases are used. Figure 7 shows that selected peptides in different colors. The selected B-cell and T-cell epitopes are combined with the help of different linkers, adjuvants, and PADRE sequences (Table 7); it helps to enhance the immunogenicity of the vaccine. These shortlisted epitopes are highly effective, nonallergen, nontoxic, immunogenic, and highly stable.

3 Results

Reverse vaccinology based on a subtractive genomic approach is employed in this current investigation to identify a novel vaccine candidate against UTI. In this study, 5192 proteins of UPEC UTI89 are identified and used for analysis.

3.1. Non-Homologous Protein Extraction

The total of 5192 proteins retrieved from UniProt were subjected to UniProt pblast with a p-value (0.0001) against H. sapiens. The BLAST result in the form of HIT or NO-HIT. The HIT results mean that the protein is homologous to human, and NO-HIT results in protein sequences are non-homologous to human. About 3840 proteins classified in NO-HIT were selected for further analysis.

3.2. Essential Gene Identification

DEG server is used for the analysis of essential genes of UPEC UTI89. To check the essential genes, we BLASTp against E. coli MG1655II (the reason behind selecting MG1655II instead of UPEC UTI89 is that UPEC UTI89 is not completely identified yet, so) we select a minimum of 80% to a maximum of 100% as essential genes; thus, 292 are selected. To validate these essential genes are present in UPEC UTI89, the result of DEG essential genes is subjected to BLASTp in UniProt against UPEC UTI89, and we determined 148 essential genes are present in UPEC UTI89. Another tool (SignalP) is used to find the essential genes of UTI89 with a probable range of p-value 0.7, but in this server only one gene more than the probable range was selected through the literature review. It is difficult to further analyze with one essential gene; that's why we eliminate the essential gene of the SignalP server result (error).

3.3. Subcellular Localization

The subcellular localization of proteins was determined through PsortB and the UniProt database. The total of 5192 out of 3840 proteins membrane-related proteins (cell outer membrane, inner membrane, cell membrane proteins, periplasmic, pilus, bacterial flagellum basal body proteins, and other GO (gene ontology) annotation-based localization proteins) 1207 are selected. Thus, the outer membrane is a good candidate for a vaccine. Therefore, from 1207 membrane-related proteins, 86 outer membrane proteins are screened for vaccine construction. Below Table 1 shows that cellular membrane proteins. And homologues and non-homologues to humans.

Table 1. *This table view shows samples of search results such as the cellular membrane proteins. And Homologues and Non-homologues to Human.*

Strain Protein NO.	Uni-P ENTRY NO.	ENTRY NAME	PROTEIN NAME	HIT	Cell Outer Membrane	cell inner membrane	cell Membrane	Bacterial flagelum basal body
3561	Q1RCB8	Q1RCB8_ECOUT	Putative membrane protein	N GO	YES	No	No	No
3608	Q1RDG2	Q1RDG2_ECOUT	IroN protein	N	YES	No	No	No
1524	Q1RDS8	Q1RDS8_ECOUT	Outer membrane protein F	N	YES	No	No	No
1712	Q1RFS9	Q1RFS9_ECOUT	Outer membrane pore protein E	N	YES	No	No	No
1329	Q1RC18	Q1RC18_ECOUT	Outer membrane protein N	N	YES	No	No	No
4757	Q1RCH8	Q1RCH8_ECOUT	Outer membrane protein W	N	GO outer membrane	No	No	No

Strain Protein NO.	Uni-P ENTRY NO.	ENTRY NAME	PROTEIN NAME	HIT	Cell Outer Membrane	cell inner membrane	cell Membrane	Bacterial flagelum basal body
2813	Q1RBW6	Q1RBW6_ECOUT	Probable tonB-dependent receptor YncD	N	YES	No	No	No

3.3.1. Screening of Protein Properties

Through the VaxElan Pipeline, proteins are shortlisted on the basis of their properties: adhesion property, non-bacterial pathogens, antigen probability, non-allergen, IEDB MHC-I protein binding prediction, TM helices, virulence factor, CTL epitope prediction, non-homology, subcellular localization, cleavage sites, stability, molecular weight, and Pi and Si values. These factors validate the potential vaccine target. VaxElan Pipeline server. Table 2 shows the result of the vaxElan Pipeline database for the screening of protein properties. Author refers to the supplementary material.

Table 2. *Table shows the result of vaxElan Pipeline database for the screening of proteins properties. Author refers to the supplementary material.*

	MLTF_ECOUT	Membrane-bound lytic murein transglycosylase F [.]				
Sequence_id	Cellular localization (TargetP)	Secretory / Non-Secretory Protein	Cleavage Sites	Non-Homology	Si Value	Pi Value
S0_sp|Q1R8H6|MLTF_ECOUT	1	0	1	1	10.85	0.6
	EMTA_ECOUT	Endo-type membrane-bound lytic murein transglycosylase A [.]				
Sequence_id	Cellular localization (TargetP)	Secretory / Non-Secretory Protein	Cleavage Sites	Non Homology	Si Value	Pi Value
S0_sp|Q1RCQ2|EMTA_ECOUT	0	1	1	1	11.5	0.64

3.3.2. Antigenic Property Analysis of Essential Outer Membrane Proteins

Antigenicity analysis is important for immunological response against antibodies. The 86 essential, non-homologous outer membrane proteins were analyzed through Vaxijen v2.0. Out of the total proteins, 71 proteins that are above the threshold value are selected. Shortlist of Proteins It is difficult to subject the 71 outer membranes to epitope prediction. On the basis of potential protein properties like probable antigen value, virulence factor, essential genes, adhesion properties, TM helices, cleavage sites, structure analysis, and molecular weight. 07 proteins are selected for further analysis Table 3.

Table 3. *Shortlisted Proteins of UPEC UTI89 Strain.*

Sr. No.	Strain Protein No.	Entry/Alpha fold db	Protein Name	Antigen	Molecular weight
1	3561	Q1RCB8	Putative membrane protein	0.8489	21802.98
2	3608	Q1RDG2	IroN protein	0.7910	79134.54
3	1524	Q1RDS8	Outer membrane protein F	0.7740	39354.32
4	1712	Q1RFS9	Outer membrane pore protein E	0.7677	39209.42

Sr. No.	Strain Protein No.	Entry/Alpha fold db	Protein Name	Antigen	Molecular weight
5	1329	Q1RC18	Outer membrane protein N	0.7604	41178.76
6	4757	Q1RCH8	Outer membrane protein W	0.7563	41178.76
7	2813	Q1RBW6	Probable tonB-dependent receptor YncD	0.7447	77254.64

3.3.3. Structure Analysis of Shortlisted Proteins

To visualize the structural significance of the proteins to identify a better vaccine candidate, PDB, SWISS-MODEL, and UniProt are used. These tools provide 3D structure to visualize alpha helices, beta sheets, coils, and bindings of proteins Figure 2.

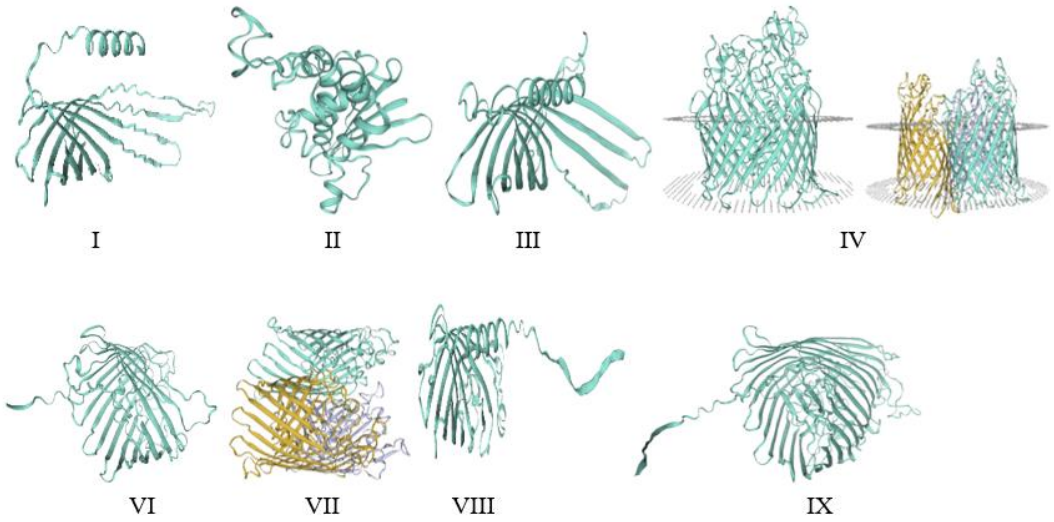
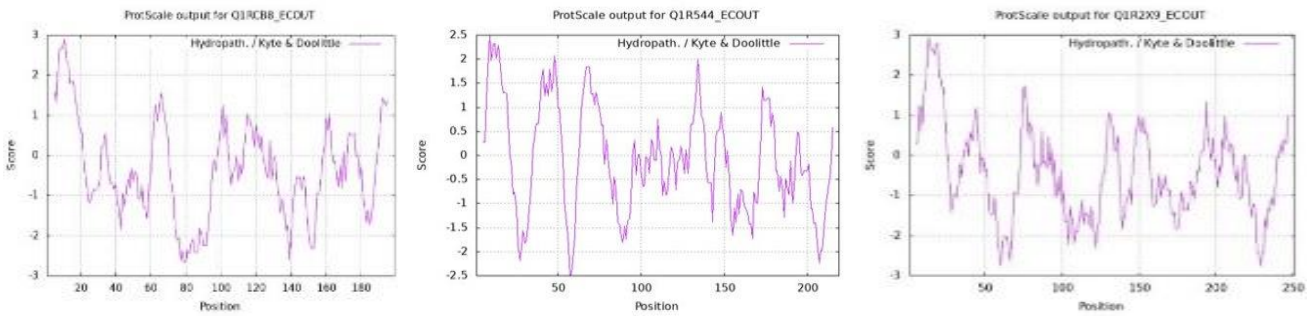


Figure 2. *Structure Analysis Shortlisted Proteins.*

3.3.4. Graphical Analysis of Hydropathy, Kyte & Doolittle

The Protscale database is used to predict the structural complexibility and inner membrane region of the proteins. The graphical analysis of hydropathy, Kyte & Doolittle, provides information on the contraction of protein structure. Figure 3 Prot Scale Database (Identification and visualization of the graphical hydropathy score)



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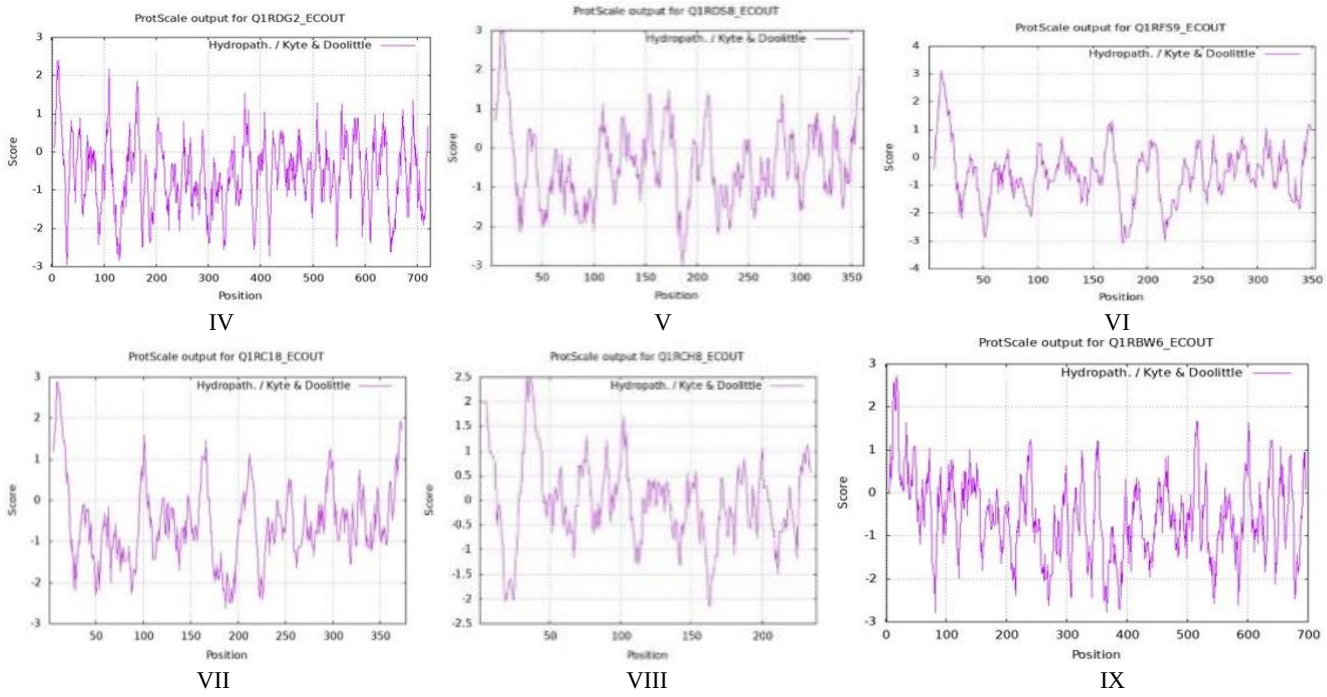
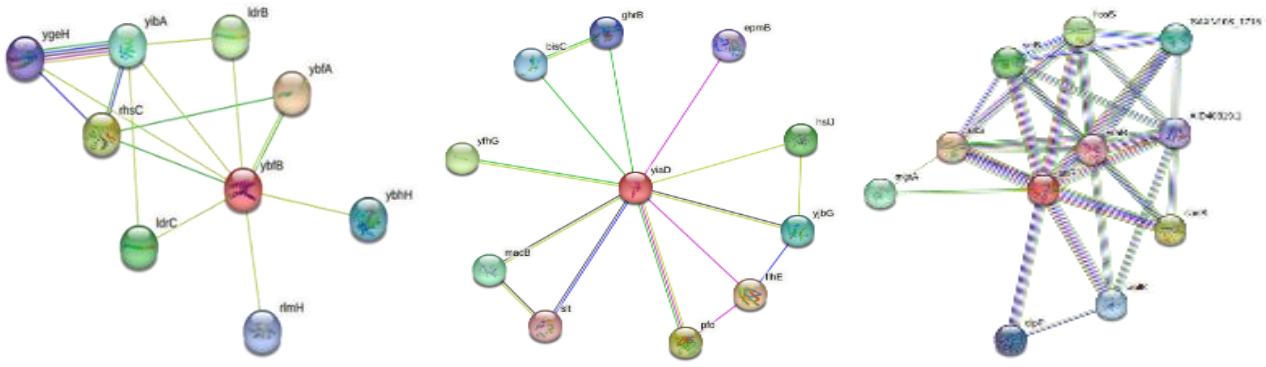


Figure 3. *Graphical analysis of Hydropathy, Kyte & Doolittle (for color representation author refers to the supplementary material) Unstability, Hydropathy, and Molecular Weight Demonstration.*

For the identification of statistical conditions where protein becomes unstable, the Expasy Protparam server is used. Hydropathy, molecular weight, and instability increase the validation of the proteins to a potential candidate. The selected proteins achieve the probable range of hydropathy, instability, and molecular weight resulted through literature review.

3.3.5. Identification of Protein-Protein Interaction

STRING database is used for further analysis of the interaction of proteins with each other, and the PPIs value summarizes the functional interaction between the proteins. It provides assessment and integration of protein-protein interactions that are both indirect (functional) and direct (physical). We should find the interaction of proteins with all of the proteins of the H. sapiens. Figure 4 STRING Data Base (Protein-Protein Interaction) (for color representation, the author refers to the supplementary material)



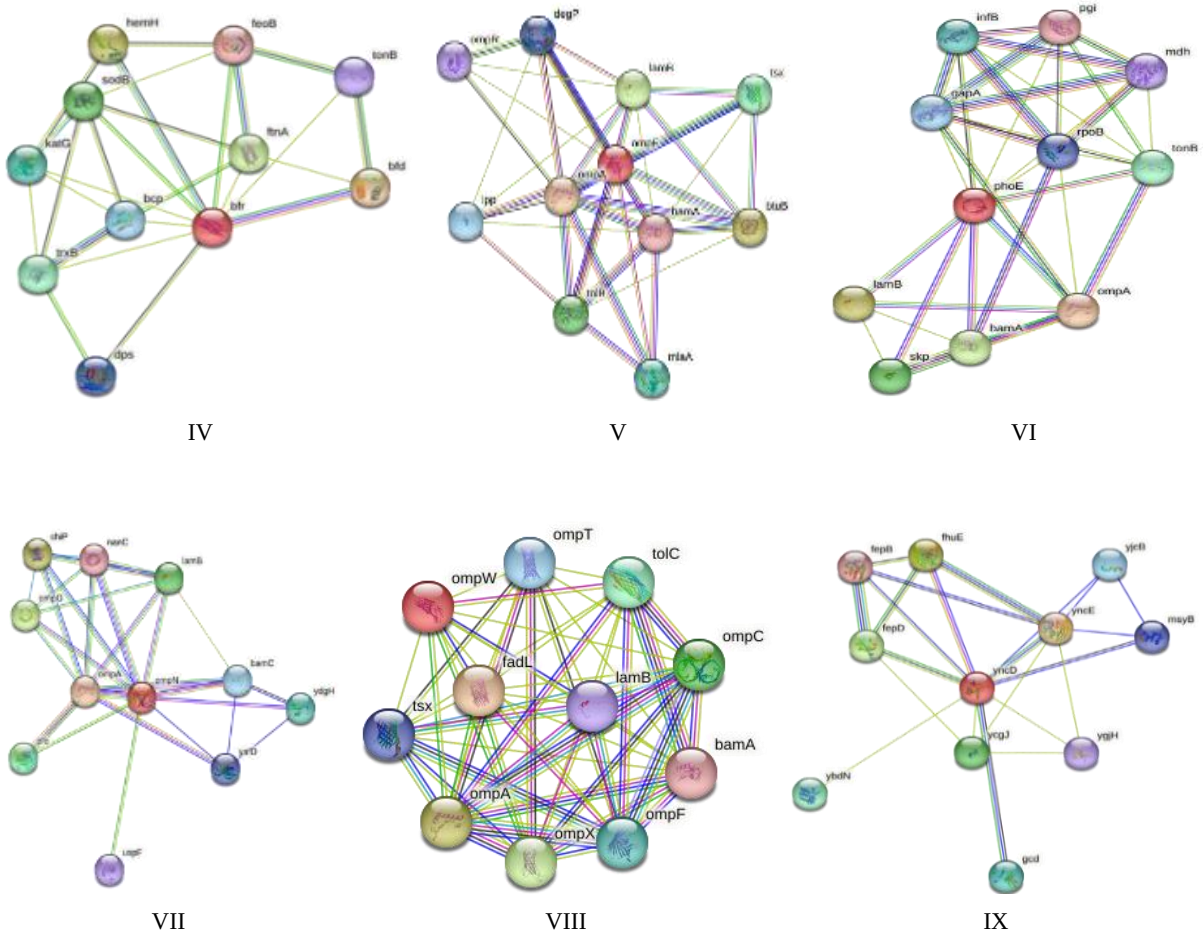


Figure 4. *STRING Data Base (Protein -Protein Interaction) (for color representation author refers to the supplementary material.*

3.3.6. Metabolic Pathways Investigation

The KEGG database is used to analyze the protein involved in which metabolic pathway, either unique or common. Commonly, metabolic pathways are those that are present in both host and pathogens, while unique pathways occur only in the pathogens. Unique metabolic pathways essential for the pathogen (E. coli) were retrieved from KEGG. (Unique pathways are not studied yet; that's why they are not found). Table 4 Common metabolic pathway of UPEC UTI89 (For Complete Pathway Author Refers to the Supplementary Material)

Table 4. *Common metabolic pathway of UPEC UTI89 (For Complete Pathway Author Refers to the Supplementary Material).*

Sr. No.	Entry	Name	Sr. No.	Entry	Name
1	eci00010	Glycolysis / Gluconeogenesis	63	eci00626	Naphthalene degradation
2	eci00020	Citrate cycle (TCA cycle)	64	eci00627	Aminobenzoate degradation
3	eci00030	Pentose phosphate pathway	65	eci00630	Glyoxylate and dicarboxylate metabolism
4	eci00040	Pentose and glucuronate interconversions	66	eci00633	Nitrotoluene degradation
5	eci00051	Fructose and mannose metabolism	67	eci00640	Propanoate metabolism
6	eci00052	Galactose metabolism	68	eci00643	Styrene degradation
8	eci00061	Fatty acid biosynthesis	69	eci00650	Butanoate metabolism
11	eci00130	Ubiquinone and other terpenoid-quinone biosynthesis	70	eci00660	C5-Branched dibasic acid metabolism

Sr. No.	Entry	Name	Sr. No.	Entry	Name
12	eci00190	Oxidative phosphorylation	71	eci00670	One carbon pool by folate
13	eci00220	Arginine biosynthesis	72	eci00680	Methane metabolism

3.4. B Cell Epitope Prediction

B cell peptides were identified using the IEDB and ABCpred, with a threshold score >0.8 used to identify the number of probable epitopes. Further analysis 07 proteins (Table 5 are selected for epitope prediction on the basis of instability, molecular weight, protein-protein interaction, metabolic pathways, and hydropathy. Additionally, to validate B cell epitope results, the tool Bepipred Linear Epitope Prediction 2.0, Chou and Fasman beta-turn prediction, Karplus and Schulz Flexibility prediction, Imini Surface Accessibility prediction, Kolasker & Tongaonkar antigenicity, and Parker Hydrophilicity prediction were used to determine the characteristics of B-cell peptides (Figure 5A-F).

Table 5. *B cell selected Epitopes.*

Sr. No.	Protein	Position	Sequence	Antigenicity
		151-165	GRHSNTSLAWGAGVQF	0.9860
1	Putative membrane protein	142-158	HDVLTGSDDGRHSNTS	1.7749
		179-195	EGSGSGDWRTDAFIVG	1.1606
		119-135	SVRYSWRGERDTRGDT	2.1718
2	iroN	655-675	TRSEDTGGLSGKELGA	1.9251
		187-203	PESSDEGATRRANFSL	1.7833
		173-203	QGNNEGASNGQEGTNNGRDVRHENGDGWGLS	2.2091
3	Outer membrane protein N	221-239	SDRTNDQVNHTAAGGDKAD	1.3568
		303-323	KGRDLHAAGGADNPAGVDDKD	1.3822
		153-169	DSTGSHDLNMRLSQQR	1.8871
4	Putative outer membrane protein	21-37	CTTNPYTGEREAGKSA	1.2357
		191-209	LGPANPIASNSTAEGKAQN	1.1965
		44-62	YFSKGNGENSYGGNGDMTY	1.7153
5	Outer membrane protein F	339-351	QIDSDNKLGVGSD	1.3620
		17-33	AGTANAAEIYNKDGNK	1.2934
		65-84	TEGAGGTLGSLGGFSVTNNT	1.1899
6	Outer membrane protein W	203-218	YMDIDTTAKYKSGVTT	1.0673
		158-178	DNGFNDHGKEAGLSDLSLKDS	0.9364
		44-72	VMSLSDQRFLSGDEEETSKYKGGDDHDTV	1.187
7	Adhesin/virulence factor Hek	167-183	TWDYEYGSSGRESLSR	1.2057
		194-210	GAGVRYDVTPDIALDL	1.8453
		175-190	QGKNENRDVKKQNGDG	2.4762
8	Outer membrane pore protein E	45-57	YMSDNDSKDGDQS	1.9845
		212-231	NSDRTNEQNLQSRGTGKRAE	1.9644
	Probable tonB-dependent receptor	479-495	SYRADGQSGMNFGLKP	1.707

Sr. No.	Protein	Position	Sequence	Antigenicity
9	YncD	679-695	YYEPAPGRNYGVGINI	1.5326
		206-217	TTHGYRDHSGAQ	1.4957

In the (Figure 5A-F, it shows that yellow colors represent the probable epitopes above the threshold value while the green ones are below the threshold value. To identify the probability of B cell epitopes Figure 5A-F, the following tools are applied to check the (A) peptide prediction, (B) available beta turns in epitope, (C) surface accessibility, (D) flexibility index, (E) antigenic values, and (F) predict the hydrophilic properties of the peptides. This IEDB software is applied to all selected protein candidates for screening.

In the Figure 5A-F, shows the prediction of *Putative membrane protein using IEDB tool*, the yellow colors represent the probable epitopes above the threshold value while the green ones are below the threshold value. See supplementary material for other proteins.

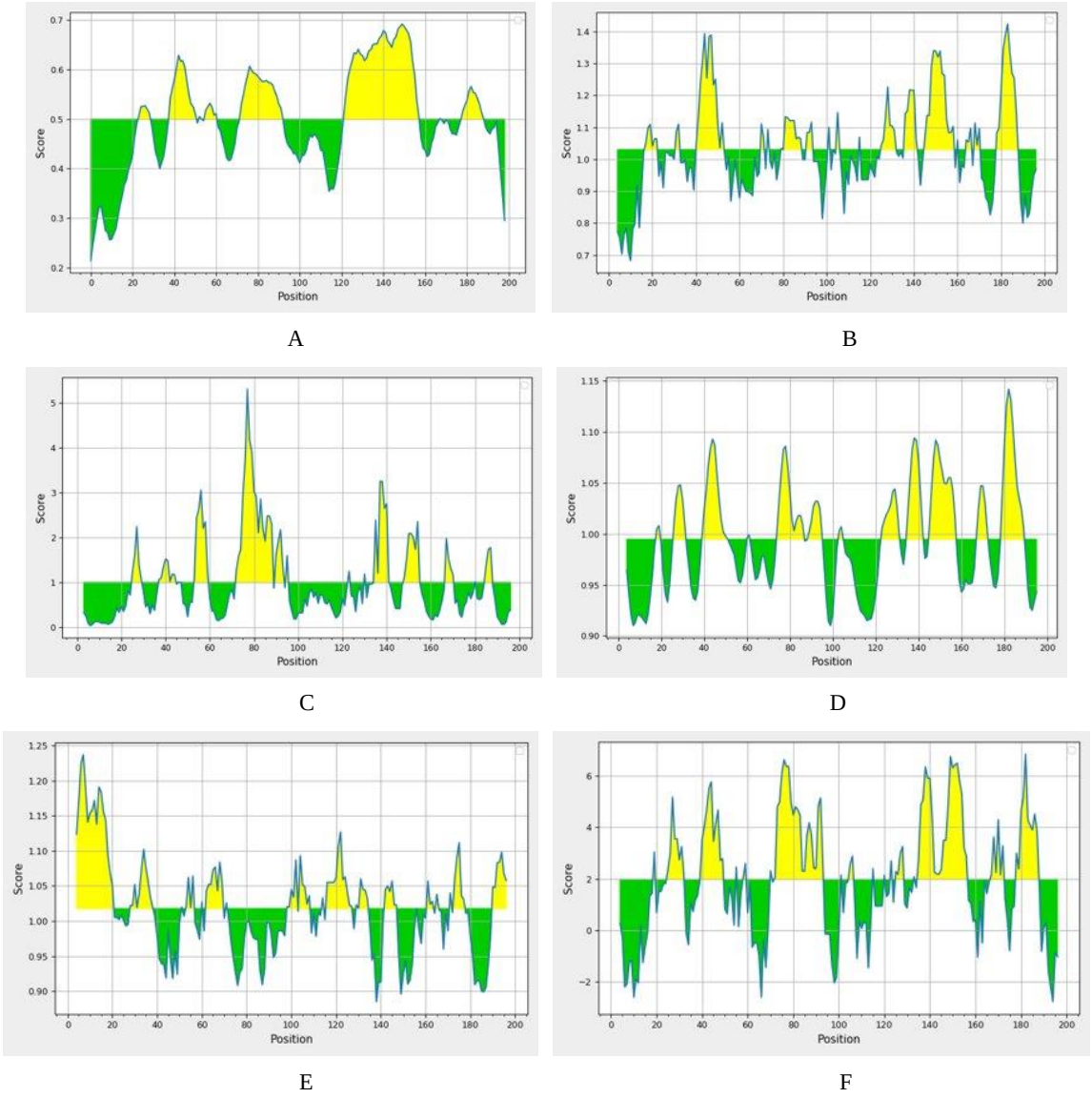


Figure 5. *Putative membrane protein (A) Epitope Prediction of Linear Bepipred, (B) Beta Turn Prediction of Chou & Fasman, (C) Imini Prediction of Surface Accessibility, (D) Schulz Flexibility and Karplus Analysis, (E) Tongaonkar and Kolaskar Antigenicity, (F) Prediction of Hydrophilicity Parker.*

3.4.1. Allergenicity and Antigenicity Prediction

The capacity to improve an immune response when an antigen is encountered by the human body. Vaxijen v.2.O was used to analyze all of the B-cell peptides, with a potential antigenic value of 0.5. For further study, epitopes with an accuracy of 89-90% and antigenic scores above the cutoff point (i.e., 0.5) were identified. To improve the efficacy of vaccine candidates, it is crucial to confirm the allergenicity of epitopes. Epitope allergenicity was evaluated using AllerTopV2.O, AllergenPRO, and AllergenFP.V.1. The predicted ratio of epitopes is <O.4 was used to determine the allergenicity of peptides.

3.4.2. MHC Class-I T Cell Peptide Prediction

The discovery of essential peptides that stimulate the immune system of the human body may aid in the establishment of memory immune cells against UPEC UTI89. The IEDB server is utilized to locate MHC class-I epitopes, and NetMHCpan-.4.1 was employed to predict MHC class-I binding. The selection of MHC-I epitopes is based on the threshold value of <0.2. By default, the HLA (Human Leucocyte Antigens) alleles are chosen to project the coverage of 97% of the global population. and the default setting for peptide length is 9mers. We use these chosen epitopes for additional analysis. Table 6 shows the shortlisted MHC-I epitopes.

Table 6. *Shortlisted MHC-I Epitopes.*

MHC 1						
Sr. No.	**Protein Name**	**Protein ID**	**Shortlisted Epitopes**	**Antigen V**	**Toxicity**	**Conservancy**
1	Putative membrane protein	Q1RCB8	LAWGAGVQF	0.7945	Non T	100%
			NLNGINVKY	2.2978	Non T	100%
2	iroN	Q1RDG2	ESSDEGATR	2.4830	Non T	100%
			RSSEGANTY	2.0478	Non T	100%
3	Outer membrane protein N	Q1RC18	AQYQFDFGL	1.6882	Non T	100%
			VLALLIPAL	1.5973	Non T	100%
4	Outer membrane protein F	Q1RDS8	AQYQFDFGL	1.6882	Non T	100%
			GLNFAVQYL	1.174	Non T	100%
5	Outer membrane protein W	Q1RCH8	SFVTPMWVY	1.5943	Non T	100%
			NTQLGLTFT	1.0300	Non T	100%
6	Adhesin/virulence factor Hek	Q1R2X9	TPDIALDLSY	1.439	Non T	100%
			SGDEEETSKY	2.3121	Non T	100%
7	Outer membrane pore protein E	Q1RFS9	AQYQFDFGL	1.6882	Non T	100%
			SVQAAEIYNK	0.8259	Non T	100%
8	Probable tonB-dependent receptor YncD	Q1RBW6	SSSGGRTTYK	2.6652	Non T	100%

3.4.3. Immunogenicity Identification of MHC-I Peptides

To analyze the immune system response, the MHC-I epitopes have been submitted to the IEDB immunogenicity prediction tool. The default parameter settings were utilized to further analyze the MHC-I epitopes less than <0.2. The selected epitopes are <0.2-0.04; the potential values retrieved by the tool were selected for further study.

3.4.4. T Cell MHC-II Epitope Prediction

The IEDB recommends the NetMHCpan2.22 approach as being crucial for the peptide prediction, which refers to >99% of the population coverage for MHC-II binding epitope prediction. Peptides are expected to be 15mers long. Additionally, MHC-II epitopes are screened using a threshold value of less than <0.2, which are selected. Table 7 validates the MHC-II selected peptides.

Table 7. MHC-II selected peptides.

MHC II					
Sr. No.	**Protein ID/UniP**	**Protein Name**	**Selected Peptides**	**MHC Alleles (IEDB)**	**Antigenicity**
1	Q1RCB8	Putative membrane protein	NRWFSVMAGPSVRVN	HLA-DRB1*01: 01	0.9785
			ITSFSYANAEDEQKT	HLA-DRB1*09: 01	0.8201
2	Q1RDG2	iroN Protein	GSGAAGGVVNIITKR	HLA-DQA1*05: 01 /DQB1*03: 01	1.2918
			GLNSQVSVAKSSDDD	HLA-DQA1*01: 02 /DQB1*06: 02	1.0814
3	Q1RC18	Outer membrane protein N	LGMGFSAGAAYTSSDR	HLA-DQA1*05: 01 /DQB1*03: 01	0.8842
			YFNKNMSTYVDYKIN	HLA-DQA1*01: 01 /DQB1*05: 01	0.6617
4	Q1R544	Putative outer membrane protein	SSATLKPAGANTLTGV	HLA-DRB1*09: 01	0.8075
			SVASALITQGVDASRI	HLA-DRB4*01: 01	0.6030
5	Q1RDS8	Outer membrane protein F	RPSIAYTKSKAKDVE	HLA-DRB5*01: 01	0.8788
			INQIDSDNKLGVGSDD	HLA-DRB1*03: 01	0.9617
6	Q1RCH8	Outer membrane protein W	ATDNIGVELLAATPF	HLA-DQA1*01: 02 /DQB1*06: 02	0.9684
			MAQWYFGDASSKFRP	HLA-DRB3*01: 01	0.8310
7	Q1R2X9	Adhesin/virulence factor Hek	KSGFYLTGKAGASVM	HLA-DRB1*08: 02	0.8799
			GKSGFYLTGKAGASV	HLA-DRB1*08: 02	0.8831
8	Q1RFS9	outer membrane pore protein E	KGKDIEGIGDEDLVN	HLA-DQA1*05: 01 /DQB1*02: 01	0.7698
			GGSDFAISGAYTNSD	HLA-DRB1*09: 01	1.0853
9	Q1RBW6	Probable tonB-dependent receptor	DDEIVVDSSSGGRTT	HLA-DRB1*04: 01	0.9475
			NAKLGVRIDDASKLS	HLA-DRB3*01: 01	0.8299

3.4.5. Allergenicity, Antigenicity, Toxicity, and Conservancy of MHC Class II Epitopes

The selected MHC-II peptides were examined for their antigenicity, toxicity, conservancy, and allergenic characteristics based on their IC50 value and percentile rank. To determine the peptides' antigenic property, which was subsequently assessed using 80-90% and a threshold of <0.5 as a likely cut-off value for probable antigen, we determined through Vaxijen v2.0. To assess an epitope's relative level of toxicity, use the ToxinPred server. Where the conservancy of peptides was predicted using the IEDB epitope conservancy analysis tool. As well as using the bioinformatic tools AllerTOP V.2.0, AllergenFP V.1.0, and AllergenPRO to predict the allergenicity of particular peptides.

3.4.6. MHC I-II Cluster Analysis

The MHC-I-II epitopes were validated and confirmed using the MHC-cluster server. Figure 6 MHCcluster-2.0 for the prediction of phylogenetic trees and heatmaps of epitopes. MHC-cluster shows the phylogenetic tree and heat map. These HLA alleles and MHC class molecules were analyzed using a cluster approach in Figure 6 heatmap clusters of MHC-I, alleles, and peptides, while in phylogenetic tree clusters of MHC-II, alleles, and peptides. The MHC clusters show the yellow portion suggests weaker binding, and the red zone indicates stronger interaction.

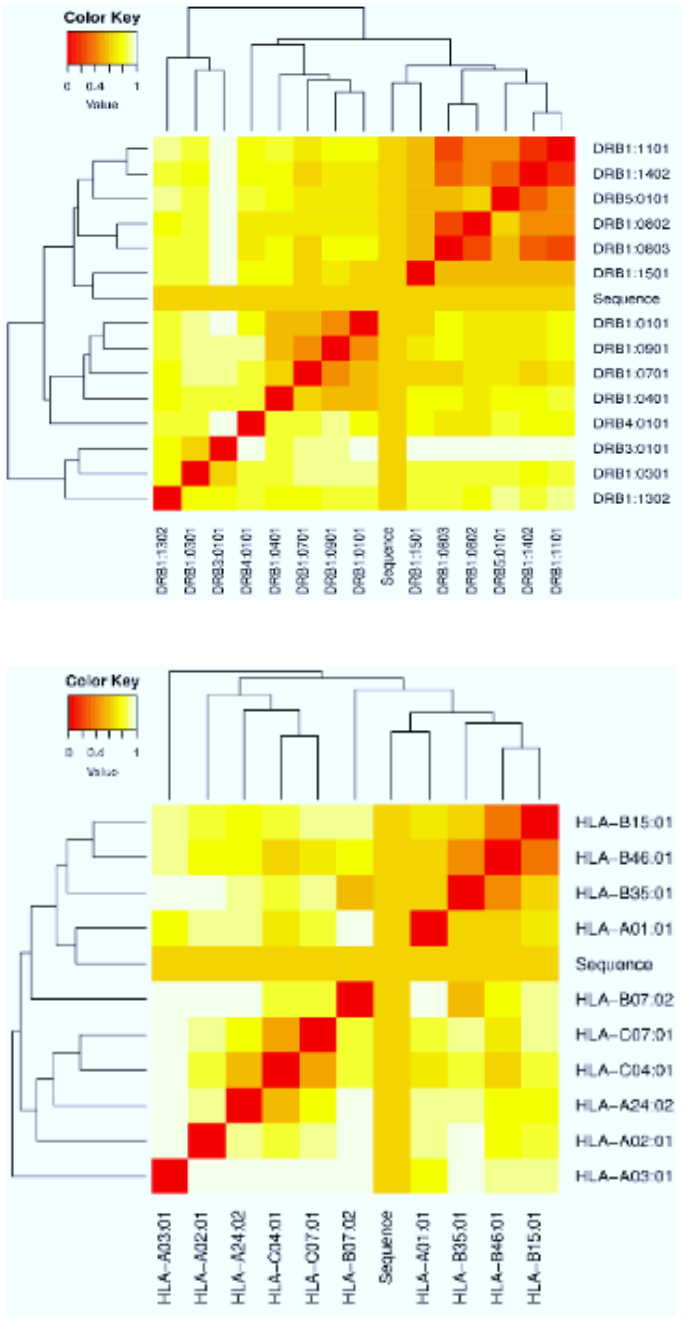


Figure 6. *Heat Map and Phylogenetic Tree.*

3.4.7. Final Epitope Mapping

After screening out the whole proteome of the strain UTI89, the selected epitopes of B and T cells are further used for vaccine construction. B cell peptides are utilized and compared with MHCI and MHCII to check out the similar sequences and make them a single epitope. There are a total of 27 B cell peptides identified; 18 peptides of MHC-I and 18 MHC-II peptides were selected from the shortlisted 07 cell outer membrane proteins. The analyzed epitopes are used for final epitope mapping and vaccine candidates using PYMOL (Figure 7), a protein visualization tool, PDB, and the Alpha Fold protein structure database. The AlphaFold Database was used to retrieve the PDB file of our shortlisted protein for the final epitope mapping of vaccine candidates. Different colors indicate the potential vaccine epitopes. We determine and summarize that these selected epitopes are the best candidates for a vaccine against UTI.

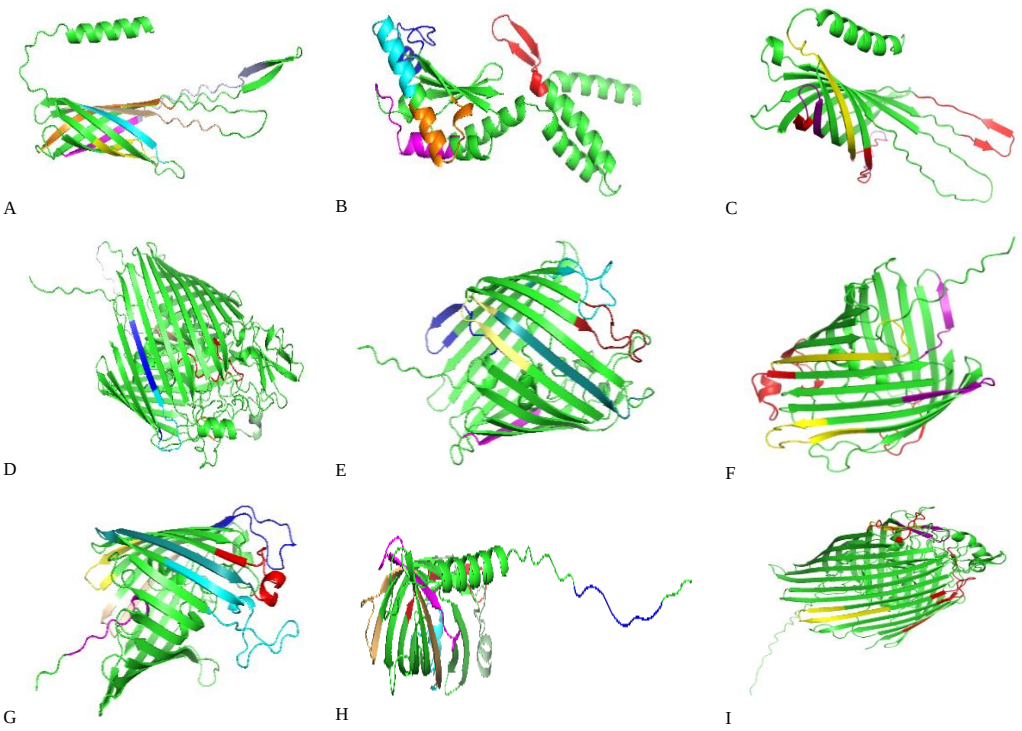


Figure 7. *Structure modeling of peptides shown by different colors obtained by (A) Alpha Fold database (B) PDB (C) Swissport Model (D) PYMOL.*

3.4.8. Final Vaccine Construct

The final epitopes were combined to form seven vaccine constructs. The vaccine construct was formed as (v1 to v7) after being combined with linkers (EAAAK, GGGS, and H-linkers (HEYGAEALERAG)) to promote the antigen presentation activity. Adjuvant, PADRE Sequence (AKFVAAWTLKAAA), the PEDRE Sequence activates the CD4+ T helper cells. Target Epitopes: All these vaccine constructs were combined through linkers (EAAAK, GGGS, HEYGAEALERAG, and AKFVAAWTLKAAA). Figure 8 shows the (V1) vaccine construct.

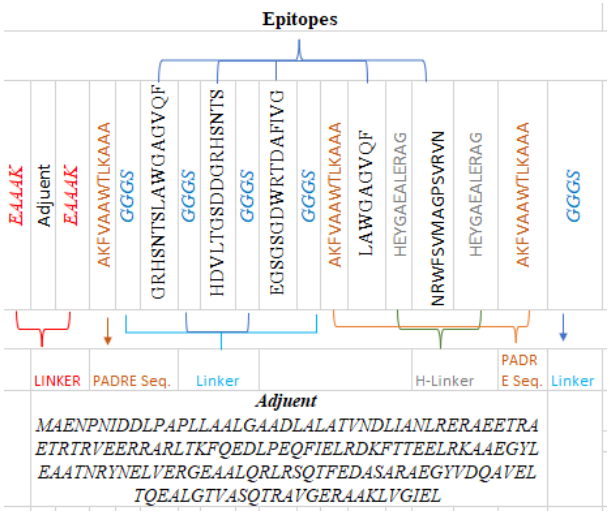


Figure 8. *of Vaccine Construct. For all Construct author refers to supplementary material.*

The vaccine constructs (v1 to v7) created from selected epitopes were assumed for physical and chemical characteristics. The aliphatic index (73.13) provides the available side chains, and the instability index (37.54) gives the confidence to the stability of the vaccine. The Vaxijen v2.0 (score > 1.115) and ANTIGENpro (score > 0.90) estimated the antigenic property of the vaccine construct. The SOLpro database is used to predict the solubility (score >0.60) and Pi value (score-5.320) of the vaccine for production. Overall, our vaccine construct is highly stable, effective, antigenic, and non-toxic. The final construct of all vaccines is shown in Table 8 given below.

Table 8. *Final Vaccine Construct with different linkers, adjuvant, and PDGRE Sequences.*

Sr. No	Vaccine Construct	Vaccine Composition with Protein Name:	Vaccine Sequence
1	v1	A0A7I7TJ71.1. A Putative membrane protein; A0A7I7TJ71_9MYCO (gene: hbhA	EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGSGRHSNTSLAWGAGVQFGGGSHDVLTGSDDGRHSNTSGGGSEGSGSGDWRTDAFIVGGGGSAKFVAAWTLKAAALAWGAGVQFHEYGAEALERAGNRWFSVMAGPSVRVNHEYGAEALERAGAKFVAAWTLKAAAGGGS
2	v2	X8B035.1. A; IroN protein AlphaFold DB model of X8B035 (gene: unknown	*EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGS*SVRYSWRGERDTRGDT*GGGS*TRSEDTGGLSGKELGA*GGGS*PESSDEGATRRANFSL*GGGSAKFVAAWTLKAAA*ESSDEGATRHEYGAEALERAGGSGAAGGVVNIITKRHEYGAEALERAG*AKFVAAWTLKAAAGGGS*
3	v3	A0A7I7TJ71.1. A 11 Outer membrane protein F, AlphaFold DB model of A0A7I7TJ71_9MYCO (gene: hbhA,	*EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGS*YFSKGNGENSYGGNGDMTY*GGGS*QIDSDNKLGVGSD*GGGS*AGTANAAEIYNKDGNK*GGGSAKFVAAWTLKAAA*GLNFAVQYLHEYGAEALERAGINQIDSDNKLGVGSDDHEYGAEALERAG*AKFVAAWTLKAAAGGGS*
5	v5	A0A7I7TJ71.1. A Outer membrane protein N AlphaFold DB model of A0A7I7TJ71_9MYCO (gene: hbhA,	*EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGS*QGNNEGASNGQEGTNNGRDVRHENGDGWGLS*GGGS*SDRTNDQVNHTAAGGDKAD*GGGS*KGRDLHAAGGADNPAGVDDKD*GGGSAKFVAAWTLKAAA*AQYQFDFGLHEYGAEALERAGLGMGFSAGAAYTSSDRHEYGAEALERAG*AKFVAAWTLKAAAGGGS*
6	v6	A0A7I7TJ71.1. A Outer membrane protein W AlphaFold DB model of A0A7I7TJ71_9MYCO (gene: hbhA,	*EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGS*TEGAGGTLGSLGGFSVTNNT*GGGS*YMDIDTTAKYKSGVTT*GGGS*DNGFNDHGKEAGLSDLSLKDS*GGGSAKFVAAWTLKAAA*SFVTPMWVYHEYGAEALERAGATDNIGVELLAATPFHEYGAEALERAG*AKFVAAWTLKAAAGGGS*
7	v7	A0A1A3EAK7.1. A Probable tonB-dependent receptor YncD AlphaFold DB model of A0A1A3EAK7_9MYCO (gene: A0A1A3EAK7_9MYCO	*EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELEAAAKAKFVAAWTLKAAAGGGS*SYRADGQSGMNFGLKP*GGGS*YYEPAPGRNYGVGINI*GGGS*TTHGYRDHSGAQ*GGGSAKFVAAWTLKAAA*SSSGGRTTYKHEYGAEALERAGDDEIVVDSSSGGRTTHEYGAEALERAG*AKFVAAWTLKAAAGGGS*

4. Discussion

Innovative development of the vaccine will be necessary to deal with the changing burden of urinary tract infections with uropathogenic *Escherichia coli*. With the advancement in genomic and proteomic data coupled with high-performance computer technologies, the investigation of peptide-based vaccines has been explored [10]. Immunotherapy is another potential approach for inducing substantial immunogenicity, safety, and cross-protection across different UPEC strains [21]. An Insilco study show that the plant derived phytocompounds were utilized to treat cancer particularly ovarian and other infections [8].

UPEC is classified as a superbug, and the CDC estimated 404.61 million infections and 236,790 deaths yearly. The antibiotic-resistant strains of UPEC pose a challenge for which effective vaccines will be needed to counter bacterial resistance [15]. Vaccination could provide prophylactic benefits with reduced occurrences of resistant infections. The current vaccine candidates designed against UPEC, including those in animal trials, need further development for increased efficacy. Although the progress is reported, not all candidates have achieved desired outcomes, thereby necessitating continued research to increase the effectiveness of a vaccine against UPEC and its related pathogens.

One recent paper applying bioinformatics that included subtractive genomics and reverse vaccinology identified both B-cell and T-cell epitopes and resulted in a multi-epitope vaccine candidate based on UPEC pathogenicity proteins. This design aimed to develop a protein-based vaccine that would elicit protective immunity. The concept incorporated MHC-I and MHC-II epitopes, as T-cell-mediated immunity is essential for stringent B-cell responses against UPEC infections [6, 23]. Thus, 3840 proteins were reported as non-homologous for UPEC, and out of these, 1207 were membrane-related, ideal targets for vaccine development. Taking all these into consideration, nine proteins were shortlisted for their antigenic potential, virulence factors, and structural properties. These are: (pmp, YiaD, vfh, iron protein, ompF, phoE, ompN, ompW, YncD).

Altogether, 18 MHC-I, 18 MHC-II, and 27 B-cell epitopes were detected across these proteins as the base for further developments for constructing vaccine research. Another strategy has already been applied for developing immunogenic T-cell vaccines against other pathogens, including Ebola [5]. The proteins identified in this study are promising vaccine candidates against UPEC. As epitope-based vaccines hold up promising prospects as a possible preventive measure against the ever-increasing antibiotic-resistant strains with subsequent positive results in public health, this study shall be seen as an important contribution to the evolution of such vaccines. Future research on the plant derived phytocompounds were utilized for the explorations and developments in this field may provide measures for the prevention of such bacteria causing UTIs.

5. Conclusions

Designing multi epitope vaccine against urinary tract infection (UTIs) represents a promising advancement in in preventing healthcare. our designed vaccine against by targeting bacteria (UPEC-UTI89) pathogens responsible for UTIs. Our multi epitope vaccine using scientific (in-silico tools) could significantly reduce the incidence of these infections, reduce the burden on healthcare systems and improve the quality of patient life. In-silico research improves the efficacy, safety, preventive aspects and broad applications of the vaccine.

Abbreviations

E. coli	*Escherichia Coli*
UTIs	Urinary Tract Infections
DALYs	Disability-adjusted Life Year
UPEC	Uropathogenic *Escherichia Coli*
HIV	Human Immunodeficiency Viruses
EPEC	*Enteropathogenic E. Coli*
EHEC	*Enterohaemorrhagic E. Coli*
ETEC	*Enterotoxigenic E. Coli*
EAEC	*Enteroaggregative E. Coli*
EIEC	Enteroinvasive *E. Coli*
DAEC	Diffusely Adherent *E. Coli*
MNEC	Meningitis-associated *E. Coli*
APEC	Avian Pathogenic *E. Coli*
AMR	Antimicrobial Resistance
IBCs	Intracellular Bacterial Communities
E. coli	*Escherichia Coli*
UTI	Urinary Tract Infections
UPEC	Uropathogenic *Escherichia Coli*
MV140	Muco-active Vaccine 140
DNA	Deoxyribonucleic Acid
EPI-752	Expanded Program on Immunization
FtsZ	Filamenting Temperature-sensitive Mutant Z
UniProt	Universal Protein Resources
NCBI	National Center of Biological Information
H. sapiens	Homo Sapiens
DEG	Database of Essential Genes
IEDB	Immune Epitope Database
MHC	Major Histocompatibility Complex
TM helix	Temperature Helix
CTL	Cytotoxic T-lymphocytes
GO	Gene Annotation
PDB	Protein Data Bank
3D	Three Dimensional
PPIs	Protein-protein Interaction
KEGG	Kyto Encyclopedia of Genes and Genomes
APCs	Antigen-presenting Cells
HLA	Human Leucocyte Antigen

PYMOL	Shown in Appendices
TCA	Citrate Cycle
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
yiaD	Putative Outer Membrane Protein
Hek	Adhesion/ Virulence Factor
iroN	Iron Protein
ompF	Outer Membrane Protein F
phoE	Outer Membrane Pore Protein E
ompN	Outer Membrane Pore Protein N
ompW	Outer Membrane Protein W
yncD	Protein Tonb-dependent Receptor

Author Contributions

Gul Zaib: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing

Saira Javed: Project administration, Supervision, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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