Genome-Wide Prediction of Potential Vaccine Candidates for *Campylobacter jejuni* Using Reverse Vaccinology

Richa Jain^{1,5} · Sarita Singh² · Santosh kumar Verma³ · Ankit Jain⁴

Received: 30 May 2017 / Revised: 1 August 2017 / Accepted: 1 September 2017 / Published online: 11 November 2017
© Springer-Verlag GmbH Germany 2017

Abstract Campylobacteriosis is a deadly disease which has developed resistance to most of the available chemotherapeutic agents. Although various studies provide evidence of acquired immunity following exposure to *Campylobacter jejuni*, no effective vaccine has been developed, still. Hence, there is an urgent need to identify potential vaccine candidates for *Campylobacter* species. In the proposed study, *Campylobacter jejuni* subsp. *jejuni* serotype O:2 (strain NCTC 11168) was taken and computational approach was employed to screen *C. jejuni* genome for promising vaccine candidates. From 1623 protein-coding sequences, 37 potential antigens were screened for epitope prediction based on surface association, consensus antigenicity predictions, solubility, transmembrane domain, and ortholog analysis. Comprehensive immunogenic analysis of these 37 antigens revealed that antigen Q0PA22 shows the greatest potential for experimental immunogenicity analysis. It has several potential CD4+ and CD8+ T-cell epitopes, as well as high probability of B-cell epitope regions as compared to well-characterized antigen

Omp18 (Uniprot ID:Q0PC24). Among the highest scoring predicted epitopes, an optimal set of epitopes with respect to overall immunogenicity in target populations for campylobacteriosis viz. Europe, North America and Southwest Asia was determined. An epitope AMLTYMQWL from antigen no. 6(Q0PA22) binds to the most prevalent allele HLA-A*0201, and this epitope has most immunogenicity for all the target populations. In addition, this epitope exhibited highly significant TCR–pMHC interactions having a joint Z value of 4.87. Homology mapping studies of the predicted epitope show best homology to a well-studied antigenic peptide from influenza virus H5N1. Therefore, the predicted epitope might be a suitable vaccine candidate.

Keywords Campylobacter · Reverse vaccinology · MHC · Epitope · Vaccine

1 Introduction

Campylobacteriosis is a collective description for infectious diseases caused by members of the bacterial genus *Campylobacter*, found in animals such as poultry, cattle, pigs, wild birds, and wild mammals. Campylobacteriosis constitutes serious medical and socioeconomic problem worldwide. *Campylobacter* bacterium is one of the major causes of foodborne diarrhoeal illness in humans, and in addition, it is the most common bacterium that causes gastroenteritis worldwide. Some virulence factors have been identified in campylobacter including flagella-mediated motility, bacterial adherence to intestinal mucosa, invasive capability, and the ability to produce toxins. [1–3]. The disease campylobacteriosis is associated with numerous sequelae, including Guillain–Barré Syndrome, inflammatory bowel disease,

Electronic supplementary material The online version of this article (doi:10.1007/s12539-017-0260-5) contains supplementary material, which is available to authorized users.

✉ Richa Jain
richa.jain917@gmail.com

- ¹ Institute of Engineering and Technology, Sitapur Road, Lucknow, India
- ² Biotech Park, Lucknow, India
- ³ Department of Environmental science and engineering, ISM dhanbad, Dhanbad 826004, India
- ⁴ India Meteorological Department, New Delhi 110003, India
- ⁵ Meteorological Office, Surmane IVRI campus, Mukteshwar, Nanital 263138, India

reactive arthritis, and irritable bowel syndrome. Guillain–Barré syndrome is a rapidly progressive acute flaccid paralysis that results from inflammatory demyelination of peripheral nerves and is the most severe and life-threatening postinfectious sequelae of campylobacteriosis [4]. Other intestinal complications include intestinal hemorrhage, perforation, ulceration, abscesses, and toxic megacolon [5]. Extra intestinal complications include meningitis, endocarditis, hepatitis, cholecystitis, osteomyelitis, pancreatitis, splenic rupture, and septic abortion [6].

At present, there are 17 species and 6 subspecies assigned to the genus *Campylobacter*. Among *Campylobacter* species, *C. jejuni* accounts for more than 92% of human infections [7, 8]. 1.3 million people are affected and approximately 76 people with *Campylobacter* infections die each year [9]. Chemotherapeutics are available for campylobacteriosis, but studies show that bacteria have developed resistance against most of them. Mainly, three mechanisms appear to be involved in antibiotic resistance in *C. jejuni*, namely, efflux of the antibiotic, mutation of the target site, and protection of the target site azithromycin and fluoroquinolones (e.g., ciprofloxacin, quinolone, ofloxacin, levofloxacin, and norfloxacin) are commonly used for treatment of these infections, but resistance to fluoroquinolones is common [9].

Circulating anti-campylobacter antibodies obtained by the experimental evidence suggests that, in humans, every primary exposure can generate an immune response and secondary exposures can enhance and contribute to that response [10]. It has been well established that prior infection with *C. jejuni* can induce protective immunity against *Campylobacter* infections in humans and animals; this strongly supports the feasibility of development of immunization-based approaches to control *Campylobacter* infections [11]. The epidemiological and experimental studies provide evidence of acquired immunity following exposure to *C. jejuni*, lending support for vaccine development [12]. Any approved vaccine for *Campylobacter* is not available until now; therefore, it is necessary to develop effective vaccines.

‘Reverse vaccinology’ is a recent approach, which starts from the genomic sequence and, by computer analysis, predicts those antigens that are most likely to be vaccine candidates [13]. Vaccine development by reverse vaccinology involves the in silico analysis of microbial genome sequences followed by the high-throughput expression of the genes of interest. Then, the recombinant proteins are used to immunize mice. The ability of the polypeptide to elicit a quantitative and qualitative immune response is assessed by analysing the post-immunization sera. This concept was first successfully applied for development of a vaccine against

serogroup B *Neisseria meningitidis* (MenB). It took less than 18 months to identify more and some novel vaccine candidates in MenB than had been discovered during the past 40 years by the conventional methods [14] and a vaccine (Bexsero®) including 3 novel antigens and outer membrane vesicles is now licensed in 30 countries [15]. Reverse vaccinology is now being applied to many bacterial, viral, and eukaryotic pathogens and has been successful in all cases in providing novel antigens for the design of new vaccines [16]. Some applications of reverse vaccinology include *Streptococcus pneumoniae*, *Porphyromonas gingivalis*, *Chlamydia pneumoniae*, *Bacillus anthracis*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Plasmodium* spp., enteropathogenic *Escherichia coli* (EPEC) and many others [17].

In the present study, *C. jejuni* subsp. *jejuni* serotype O:2 (strain NCTC 11168) which is known as major cause of campylobacteriosis was undertaken to characterize its antigens as potential vaccine candidates.

2 Materials and Methods

2.1 Retrieval of Proteome data sets

Complete sequenced proteome of *C. jejuni* subsp. *jejuni* serotype O:2 (strain NCTC 11168) consisting of 1623 protein-coding sequences has been retrieved from Uniprot database (<http://www.uniprot.org/>). A well-characterized, immuno-dominant antigen ‘omp18’ (Uniprot id Q0PC24) was also included as control to compare and validate the outcome of study. This known antigen had previously tested and verified in various experimental studies and was reported as capable of eliciting high immune response [18].

2.2 Vaccine Candidate Characterization and Epitope Prediction

Genome-wide characterization of the potential vaccine candidates has been carried out using various computational tools as described below. Initially, the *C. jejuni* proteome has been screened for surface-associated proteins with the use of SignalP and SecretomeP tools. SignalP server predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms. It is a purely neural network-based method [19]. The SecretomeP 2.0 server produces ab initio predictions of non-classical, i.e., not signal peptide triggered protein secretion. It is also based on artificial neural networks [20]. Then, consensus antigenicity predictions have been carried out using Vaxijen and ANTIGENpro

servers. Vaxijen tool was developed for antigen classification solely based on the physiochemical properties of proteins without recourse to sequence alignment [21], and ANTIGENpro is a sequence-based, alignment-free and pathogen-independent predictor of protein antigenicity [22]. Finally, selected antigens have been further characterized based on solubility predictions using ESPRESSO, a sequence-based predictor for estimating protein expression and solubility [23], transmembrane domain predictions using TMHMM server based on a hidden Markov model [24], and BLAST for ortholog analysis [25].

2.2.1 Epitope Prediction

B-cell epitope prediction has been carried out using IEDB B-cell epitope prediction tools. A collection of methods to predict continuous linear B-cell epitopes and amino acid scales, are available at IEDB <http://tools.immuneepitope.org/bcell>. These tools predict regions of proteins that are likely to be recognized as epitopes in the context of a B-cell response. Furthermore, CTL epitope prediction has been carried out using NetCTL tool. It is artificial neural network-based tool and does so by integrating predictions of peptide MHC class I binding, proteasomal C terminal cleavage, and TAP transport efficiency. NetCTL provides a comprehensive prediction about epitopes binding to 12 HLA class I supertypes including 5 HLA-A [A1, A2, A3, A24, A26] and 7 HLA-B [B7, B8, B27, B39, B44, B58, B62] [26]. To predict binding of peptides to HLA-DR, HLA-DQ, HLA-DP, and mouse MHC class II alleles, Net-MHCII 2.2 server has been used. It is based on artificial neural networks [27].

2.2.2 Population Coverage Analysis and pMHC–TCR Interaction Analysis

OptiTope tool has been used to determine the optimal set of epitopes from among the predicted epitopes, which will yield the best immune response in the target populations/geographic areas for the campylobacteriosis viz. Europe, North America and Southwest Asia. OptiTope deployed three widely used MHC class I binding affinity prediction methods, namely, BIMAS, SYFPEITHI and SVMHC [28].

Furthermore, the PAComplex server has been employed to predict TCR–pMHC interactions of a target protein or a set of peptides and search template-based homologous peptide antigens for the query protein sequence. This server first identifies significantly similar TCR–pMHC templates (joint

Z value ≥ 4.0) of the query using antibody–antigen and protein–protein interacting scoring matrices for peptide–TCR and pMHC interfaces, respectively [29]. The joint Z value (J_z) is defined as

$$J_z = \sqrt{Z_{\text{MHC}} \times Z_{\text{TCR}}}$$

[30]. Here, $J_z \geq 4.0$ is considered a significant similarity according to the statistical analysis.

PAComplex then identifies the homologous peptide antigens of these hit templates from complete pathogen genome databases and experimental peptide databases. Finally, the server outputs peptide antigens and homologous peptide antigens of the query and displays detailed interacting models of hit TCR–pMHC templates. Here, the CTL epitope set predicted by NetCTL and optimized by OptiTope for the different target populations has been used as the target peptide set and TCR–pMHC interactions have been analyzed.

2.2.3 Epitope Homology Mapping

The IEDB homology mapping tool maps linear epitopes to 3D structures of proteins [31]. This is done by comparing the epitope source protein sequence with that of proteins with known 3D structures in the PDB. The tool generates an alignment between the target sequence of the epitope source sequence and a homologous sequence from the PDB, and visualizes the result in an Epitope Viewer.

3 Results and Discussion

Campylobacter jejuni infection is commonly self-limiting, but persistent disease can occur, and in addition, a number of chronic sequelae have been identified. The fact that recovery from a primary infection renders the host resistant to subsequent infections indicates that a vaccine against *Campylobacter* is feasible. In this study, genome-wide screening was done using various computational tools that provide an in hand idea to predict and design new vaccine and vaccine components.

3.1 Selection of Antigens and Their Characterization

The entire protein repertoire of *C. jejuni*, consisting of 1623 protein-coding sequences, has been screened for proteins with signal peptides, which account for secretion of protein through classical pathway as well as for proteins which secrete through non-classical pathway. Out of these 1623, 113 proteins have been found to contain signal peptides, using SignalP tool (Table S1) and 143 proteins have been

found to be secreted through non-classical pathway in the cell, using SecretomeP tool (Table S2). Thus, total 256 secretory proteins have been selected.

Further screening involves antigenicity predictions of these 256 proteins using VaxiJen and ANTIGENpro tools. Predefined threshold of 0.6 has been applied for both the tools as this value provide results with better accuracy, sensitivity, and specificity [21, 22]. In this analysis, 60 proteins have shown antigenic probability ≥ 0.6 ; therefore, based on the consensus predictions, these 60 antigenic proteins have been taken for further analysis (Table 1). Control antigen Omp18 (Uniprot Id Q0PC24) has been predicted as non-classically secreted protein using SecretomeP tool. It has been found to be antigenic by both VaxiJen and ANTIGENpro tools.

Protein insolubility has been known to be a major obstacle for many experimental studies; thus, 60 antigenic proteins predicted in the previous step have been subjected to predict the propensity of a protein to be soluble on overexpression using ESPRESSO tool. Out of the 60 antigenic proteins, 45 have been predicted to be soluble on overexpression and control antigen Omp18 has been predicted to be soluble on overexpression (Table S3). In transmembrane domain prediction of these 60 proteins using TMHMM server, only 1 antigen has been predicted to contain two transmembrane regions, 8 antigens (nos. 11, 18, 21, 32, 38, 43, 47, 51 in Table S3) with one, while rest antigens have been found to contain no TM regions (Table S3). The control antigen Omp18 has also not shown any TM regions. An issue in vaccine development is the possibility that vaccine candidates showing sequence identity with the host (e.g., human or mouse) proteins are likely to cause autoimmunity in the host, and thus should be discarded to avoid potential autoimmunity. In this study, potential orthologs in the human and mouse host have been searched using reciprocal BLASTP. Out of the selected 45, 8 antigens (nos. 13, 24, 27, 30, 36, 40, 43, 50 in Table S3) show orthologs in human and mouse. These may be predicted to have the potential of generating autoimmune response and, thus, are discarded for the further analysis. Based on screening so far, finally, 37 candidate antigens have been selected for epitope prediction (Table 2).

3.2 Epitope-based analysis of the selected antigens

A high probability of B-cell epitope regions has been observed in eight antigens (nos. 3, 6, 11, 12, 24, 26, 31, and 37 in Table 2) using linear B-cell epitope prediction methods. Among these, antigen no. 6 (Q0PA22) has been found to show the highest probability in the region ranging from 65 to 80 and 150 to 165, as shown in Fig. 1a–f (in each graph, the yellow area above the threshold depicts the region of high propensity of the particular physico-chemical property to which the method corresponds). These regions have been

Table 1 List of proteins predicted to be antigenic with corresponding antigenic probabilities

Protein no.	Uniprot id	Antigenic Probability	
		VaxiJen	ANTIGENpro
1	Q0P9Q4	0.6028	NA
2*	Q0P8E9	0.6043	0.948972
3	Q9PIQ1	0.6057	NA
4*	Q0P7V9	0.609	0.750863
5*	Q0PBX9	0.6092	0.864711
6	Q0PBW0	0.6096	NA
7	Q0P9Z0	0.6104	NA
8*	Q0P901	0.6104	0.722062
9*	P80672	0.6142	0.722752
10*	Q0PC85	0.6142	0.828451
11*	Q0PBR9	0.6152	0.612435
12*	Q0PAI1	0.6177	0.907918
13	Q0P7Q8	0.6209	NA
14*	Q0PA21	0.625	0.641823
15*	Q0PBH3	0.6358	0.934759
16*	Q0PAD8	0.6378	0.872639
17*	Q9PIR0	0.6394	0.949683
18*	O69302	0.6403	0.888151
19	Q0PBD5	0.6475	NA
20	Q0PBW3	0.6475	NA
21*	Q9PLX1	0.6476	0.862718
22*	Q0P8Y7	0.6484	0.605215
23*	Q46125	0.6502	0.705779
24*	Q0P7X0	0.6505	0.843714
25*	Q0P8M7	0.6505	0.861401
26*	Q0P986	0.6514	0.666757
27	P45492	0.6514	NA
28*	Q0P8M8	0.655	0.66418
29	Q0PA70	0.6555	NA
30*	Q0PC83	0.6572	0.94006
31	Q0P937	0.66	NA
32*	Q0PBB9	0.6606	0.945148
33	Q0PBV8	0.6633	NA
34*	Q9PII8	0.6641	0.68324
35*	Q9PLX6	0.6725	0.809367
36*	Q0PA22	0.6772	0.609157
37*	O85213	0.6783	0.837565
38*	Q0PC23	0.6809	0.910274
39*	Q0P7X2	0.6842	0.821075
40	Q0PAE1	0.6852	NA
41*	Q9PLX4	0.6856	0.704542
42*	Q0P8M0	0.6876	0.695071
43*	Q0P8Q1	0.6954	0.957489
44*	Q0P9S7	0.6956	0.857222
45*	Q9PPM0	0.6974	0.934889
46*	Q0PA11	0.6976	0.95496
47	Q9PIP2	0.6985	NA

Table 1 (continued)

Protein no.	Uniprot id	Antigenic Probability	
		VaxiJen	ANTIGENpro
48*	Q0P7V2	0.7041	0.942272
49*	Q0P8X7	0.7054	0.844563
50*	Q9PHW6	0.7146	0.938164
51*	Q0PAR1	0.7168	0.92021
52*	P56964	0.7211	0.926111
53	Q9PLY2	0.7212	NA
54	Q0P9W3	0.7357	NA
55*	Q9PMI3	0.736	0.681929
56	Q0PB93	0.7372	NA
57*	Q0PAN9	0.7387	0.949942
58*	Q0P7Z7	0.7397	0.831528
59*	Q0PBN7	0.7458	0.914245
60*	Q0P7T9	0.7475	0.683849
61*	P56963	0.7571	0.915357
62*	Q0PC44	0.7782	0.884757
63*	Q0P7X5	0.7783	0.681143
64*	Q9PMJ8	0.7886	0.843072
65*	Q0P7Q2	0.7888	0.939832
66	Q0PAE0	0.79	NA
67*	Q0PAS3	0.7902	0.63537
68*	Q0PBA6	0.7931	0.95115
69	Q0P7U2	0.7958	NA
70*	Q0P9T0	0.7993	0.907989
71*	Q0PAS5	0.8014	0.899646
72*	Q9PI58	0.8297	0.67936
73*	Q0PB90	0.8359	0.636843
74*	Q0P914	0.8535	0.741765
75	Q0P8F1	0.8564	NA
76*	Q0PB82	0.8586	0.659147
77	Q0P9N1	0.8672	NA
78	Q0PBX0	0.8707	NA
79	Q9PIX2	0.8766	NA
80*	Q0PAW9	0.8807	0.919957
81	Q9PIG9	0.8818	NA
82	Q0PA72	0.8957	NA
83	Q0P8R1	0.9025	NA
84	Q0PA31	0.9818	NA
85*	Q0PB92	1.0362	0.884229
86*	Q0PC84	1.0657	0.967576
87	Q9PNX4	1.0733	NA
88	Q9PI38	1.1964	NA
89	Q9PHM1	NA	0.961057
90	Q0PA96	NA	0.952036
91	Q0P8G5	NA	0.942712
92	Q9PID1	NA	0.938776
93	P96747	NA	0.931745
94	Q0P9T3	NA	0.92947
95	Q0PAA7	NA	0.929047

Table 1 (continued)

Protein no.	Uniprot id	Antigenic Probability	
		VaxiJen	ANTIGENpro
96	Q0PC45	NA	0.928969
97	Q0PBA4	NA	0.921329
98	Q0P8Q2	NA	0.916345
99	Q0PA76	NA	0.913673
100	Q0PAI2	NA	0.913256
101	Q0PAH9	NA	0.911741
102	Q0PBW9	NA	0.906708
103	Q0PAY8	NA	0.906706
104	Q0PAE2	NA	0.9005
105	Q0PC46	NA	0.896823
106	Q0PAD4	NA	0.892556
107	Q0PB95	NA	0.879982
108	Q0PAV7	NA	0.876737
109	Q0P9T1	NA	0.876657
110	Q0P967	NA	0.875618
111	Q0P9M6	NA	0.868266
112	Q0PBW1	NA	0.865464
113	Q0PCC0	NA	0.864433
114	Q9PHY8	NA	0.860455
115	Q0P802	NA	0.857528
116	Q0PA41	NA	0.845508
117	Q0P970	NA	0.844061
118	Q0PC26	NA	0.841645
119	Q0PCA5	NA	0.84138
120	Q0PAB4	NA	0.840881
121	Q0PB96	NA	0.83628
122	Q0P7Z8	NA	0.82534
123	Q0P941	NA	0.824701
124	Q0PBH6	NA	0.823866
125	Q0PAY9	NA	0.82338
126	Q0PBZ2	NA	0.816098
127	Q0P8W8	NA	0.810707
128	Q9PI35	NA	0.809116
129	Q0P8C8	NA	0.795077
130	Q9PM82	NA	0.790075
131	Q0PC89	NA	0.789636
132	Q9PLX2	NA	0.787269
133	Q0PAS1	NA	0.784577
134	Q0P928	NA	0.783668
135	Q0PBR2	NA	0.781652
136	Q0PBE5	NA	0.781205
137	Q0P814	NA	0.780918
138	Q0P8P4	NA	0.779281
139	Q0PC56	NA	0.777948
140	Q0PBE3	NA	0.772361
141	Q0PBL7	NA	0.770826
142	Q0PAU9	NA	0.770272
143	Q0PAE3	NA	0.768064

Table 1 (continued)

Protein no.	Uniprot id	Antigenic Probability	
		VaxiJen	ANTIGENpro
144	Q0PAQ4	NA	0.759686
145	Q9PI34	NA	0.757501
146	Q0P963	NA	0.757383
147	Q0PBP1	NA	0.75392
148	Q9PJ14	NA	0.753903
149	Q0PC77	NA	0.753814
150	Q0P8F9	NA	0.751982
151	Q0P8H0	NA	0.750958
152	Q0PAX6	NA	0.74712
153	Q0P9N4	NA	0.746667
154	Q0P956	NA	0.744434
155	Q0PA75	NA	0.742188
156	Q0PAE4	NA	0.7407
157	Q0PC57	NA	0.730971
158	Q0P9F8	NA	0.728406
159	Q0P7W1	NA	0.719936
160	Q0PBY5	NA	0.715185
161	Q0PBT9	NA	0.707932
162	Q0PB86	NA	0.692799
163	Q0PBF1	NA	0.688843
164	Q0PBT7	NA	0.68668
165	Q0PB85	NA	0.685236
166	Q9PNB9	NA	0.685118
167	Q9PLX5	NA	0.680692
168	Q0PA26	NA	0.680242
169	Q0P8M9	NA	0.669246
170	Q0PAG4	NA	0.668708
171	Q9PIC3	NA	0.665268
172	Q0P987	NA	0.661024
173	Q0P904	NA	0.659951
174	Q0PBZ4	NA	0.656962
175	Q0PA74	NA	0.644487
176	Q0PAF1	NA	0.626031
178	Q0P885	NA	0.62296
179	Q0P7S6	NA	0.621245
180	Q0PAF2	NA	0.62011
181	Q0PAI5	NA	0.619895
182	Q46121	NA	0.60753
Omp18	Q0PC24	0.96	0.931913

*Proteins which are common in both VaxiJen and ANTIGENpro tools

predicted to elicit significant humoral immune response, thus, the probable region for B-cell epitope.

T-cell epitope predictions have also been performed for the selected 37 antigens using NetCTL and NetMHC II program. For HLA class I supertypes, based on the highest

Table 2 List of selected 37 candidate antigens

Antigen no.	Uniprot id
1	Q0P8E9
2	Q0PBX9
3	Q0P901
4	Q0PC85
5	Q0PBR9
6	Q0PA22
7	Q0PBH3
8	Q0PAD8
9	O69302
10	Q9PLX1
11	Q0P8Y7
12	Q46125
13	Q0PC83
14	Q0PBB9
15	Q9PLX6
16	Q0PA21
17	Q0PC23
18	Q0P8M0
19	Q0P8Q1
20	Q9PPM0
21	Q9PHW6
22	Q0PAR1
23	Q0P7Z7
24	Q0P7T9
25	Q0PC44
26	Q0P7X5
27	Q9PMJ8
28	Q0PBA6
29	Q0P9T0
30	Q0PAS5
31	Q9PI58
32	Q0PB90
33	Q0P914
34	Q0PB82
35	Q0PAW9
36	Q0PC84
37	Q0PB92
Omp18	Q0PC24

value of combined score obtained using NetCTL, a total of 2512 putative CTL epitopes have been predicted, including 1217 HLA-A (247 A1, 245 A2, 328 A3, 156 A24, 241 A26) (Table S4a) and 1295 HLA-B (123-B7, 170-B8, 128-B27, 189-B39, 131-B44, 153-B58, 401-B62) (Table S4b) supertype binding peptides. Antigen no. 6 has been found to show greater number of potential CD4 + and CD8 + T-cell epitopes, as compared to well-characterized antigen Omp18

Fig. 1 **a** Chou and Fasman beta turn prediction for antigen no. 6. **b** Emini surface accessibility prediction for antigen no. 6. **c** Karplus and Schultz flexibility prediction for antigen no. 6. **d** Kolaskar and Ton-

gaonkar antigenicity prediction for antigen no. 6. **e** Parker hydrophobicity prediction for antigen no. 6. **f** Bepipred linear epitope prediction for antigen no. 6

(Uniprot ID:Q0PC24). It has been shown a total of 56 putative CTL epitopes, including 28 HLA-A (5-A1, 7-A2, 8-A3, 3-A24, 5-A26), and 28 HLA-B (1-B7, 9-B8, 5-B27, 2-B39, 2-B44, 4-B58, 5-B62), while the control Omp18 showed

a total of 33 putative CTL epitopes, including 15 HLA-A (3-A1, 4-A2, 5-A3, 3-A24, 0-A26), and 18 HLA-B (1-B7, 5-B8, 2-B27, 1-B39, 2-B44, 3-B58, 4-B62) as shown below from Table S4a, b, which is significantly lower than

antigen no. 6. For HLA class II supertypes, a total of 371 putative HTL epitopes have been predicted using NetMHC II algorithm (Table S5a, b). The control antigen Q0PC24 has shown nine allelic epitopes. Overall, test antigen no. 6 has been found to show the highest number of supertype epitopes and thus has been predicted as best antigen.

3.2.1 Selection of Optimal Epitope Sets Based on Population Coverage Analysis

Epitope vaccines trigger an immune response by confronting the immune system with immunogenic peptides. Binding of these peptides to proteins from the major histocompatibility complex (MHC) is crucial for immune system activation. However, since the MHC is highly polymorphic, crucial step in design of a peptide vaccine is the selection of the set of epitopes which yields the best immune response in a given population or individual. Hence, key criteria for selection are, e.g., overall immunogenicity, tolerance for antigen mutations, population coverage, and antigen coverage. It has been demonstrated that a correlation exists between immunogenicity and MHC class I binding affinity [32]. It is, therefore, reasonable to use MHC class I binding affinity prediction methods for the prediction of immunogenicity. Table 3 shows results of population coverage analysis for target populations of campylobacteriosis. The epitope AMLTYMQWL from antigen no. 6 binds to allele A*0201 which have the highest frequency (27.14%) in the population of Europe, the second highest frequency (14.48%) in the population of North America, and the highest frequency (15.8%) in the population of Southwest Asia.

3.2.2 TCR–pMHC Interaction Analysis

Antigen presentation and recognition occurs in two steps. The peptide ligand first binds to the major histocompatibility complex (MHC) molecule, then recognition of this peptide–MHC

(pMHC) complex by the T-cell receptor (TCR). Thus, adaptive immune response is triggered by specific T-cell receptors (TCR) binding to pMHC complexes. Here, the CTL epitope set predicted by NetCTL and optimized by Optitope for the different target populations has been used as the query peptide set and TCR–pMHC interactions have been analyzed. In TCR–pMHC interactions, the same hit peptide antigens have been obtained for all the three target populations. The epitope AMLTYMQWL has been found to have a joint Z value of 4.87, indicating that this peptide exhibits highly significant TCR–pMHC interactions (Fig. 2).

This hit peptide is homologous to template peptide GILGFVFTL, which is an epitope of matrix protein 1 of Influenza A virus as recorded in IEDB (Table 4). Hit peptide antigen has been obtained for antigen no. 6 Q0PA22, and in addition, it gave the best hit peptide for all the three populations; thus, it has high probability of exhibiting good pMHC–TCR interactions. Hence, it has been inferred as the most potential antigen.

3.2.3 Epitope Homolog Mapping

Using the 3D structural homology mapping tool, it becomes possible to determine the peptide sequence in a folded protein that would most closely depict the structure of a short peptide in solution. The top hit peptide AMLTYMQWL is homologous to template peptide GILGFVFTL as obtained by TCR–pMHC interaction analysis in the previous step. The peptide family of the template obtained contains 43 homologous peptide antigens from 27 organisms. Amongst these 43 homologous peptide antigens, the best homologous peptide GMLGFVFTL gave 3 PDB hits, and hence, it has been mapped to 3D structures of proteins available at PDB, using the homology mapping tool.

This best homologous peptide GMLGFVFTL exhibits the greatest homology with the peptide 58GILGFVFTL66

Table 3 The population coverage analysis of optimized CTL epitopes for different target populations

Target population	Covered alleles/target alleles	Population coverage (%)	Epitope	Fraction of overall immunogenicity	Covered alleles
Europe	18/19	99.6	VLLFVFFAV	0.12	A*0201
			AMLTYMQWL	0.07	A*0201
North America	16/16	99.23	VYYKKSSSL	0.12	A*2402 Cw*0401
			AMLTYMQWL	0.04	A*0201
South-west Asia	18/19	96.23	VLLFVFFAV	0.12	A*0201 A*0205
			AMLTYMQWL	0.07	A*0201 A*0205
Control antigen Q0PC24					
Europe	9/19	81.99			
North America	7/16	75.86			
South-west Asia	4/8	65.97

Fig. 2 PACOMPLEX server showing pMHC–TCR interactions for the template peptide (GILGFVFTL)

(labeled in Fig. 3) from matrix protein 1 of influenza A virus ACROWKYTOT12004 (H5N1) (chain A) (PDB ID: 2z16). It shared 99% sequence identity, with E value 5.63E-85. It has also been found to share eight out of nine identical

residues with the peptide 58GILGFVFTL66. The residues 58–66 adopt a helix conformation, as evident from Fig. 3.

The homology mapping studies of the predicted epitope have been shown the best homology to the matched primary

Table 4 List of the top 5 hit CTL peptides for the three target populations, with their corresponding joint Z value and homologous template peptide

Population	Rank	Peptide sequence	Joint Z value	Best template	Template peptide	No. of identical residues
Europe	1	AMLTYMQWL	4.87	2vlk	GILGFVFTL	2/9
	2	RMAELTTYL	3.4	3d3v	LLFGFPVYV	1/9
	3	VLLFVFFAV	3.16	2j8u	ALWGFFPVL	2/9
	4	YMQWLSQGV	1.63	2p5w	SLLMWITQC	0/9
	5	AMGDNIFAA	1.58	1qrn	LLFGYAVYV	0/9
North America	1	AMLTYMQWL	4.87	2vlk	GILGFVFTL	2/9
	2	RMAELTTYL	3.4	3d3v	LLFGFPVYV	1/9
	3	VLLFVFFAV	3.16	2j8u	ALWGFFPVL	2/9
	4	YMQWLSQGV	1.63	2p5w	SLLMWITQC	0/9
	5	AMGDNIFAA	1.58	1qrn	LLFGYAVYV	0/9
South-west Asia	1	AMLTYMQWL	4.87	2vlk	GILGFVFTL	2/9
	2	RMAELTTYL	3.4	3d3v	LLFGFPVYV	1/9
	3	VLLFVFFAV	3.16	2j8u	ALWGFFPVL	2/9
	4	YMQWLSQGV	1.63	2p5w	SLLMWITQC	0/9
	5	AMGDNIFAA	1.58	1qrn	LLFGYAVYV	0/9

Fig. 3 3D image of the matrix protein 1 of influenza A virus showing the homologous peptide 58GILGFVFTL66, in magenta

sequence of matrix protein 1 of influenza A virus ACROW-KYOTOT12004 (H5N1) (chain A), which is a well-known antigen inducing potential immune responses in the human host. Thus, 3D homology mapping increases the likelihood that a T-cell receptor raised against the peptide would be able to recognize, or cross-react with the corresponding epitope in the folded protein.

Keeping in mind the above observations, it can be deduced that the epitope AMLTYMQWL from antigen no. 6 (Q0PA22) has emerged as a promising vaccine candidate in this prediction strategy, and it can be considered to be capable of eliciting significant immune responses in experimental study so it should undergo further *in vivo/in vitro* investigations.

4 Conclusion

Vaccines elicit immune responses, provide protection against microorganisms, and are considered as one of the most successful medical interventions against infectious diseases. The development of an effective and affordable vaccine against campylobacteriosis is crucial for global public health. With the help of available and emerging epitope mapping tools, it is now possible to screen a whole proteome *in silico*, followed by advanced focus on the resulting sets of peptides. The prediction and analysis of HLA-binding sequences within protein antigens are fundamental to modern vaccine development. Nowadays, reverse vaccinology is being applied to many bacterial, viral, and eukaryotic pathogens and has shown to be of immense importance in all cases in providing novel antigens for the design of new

vaccines. The present study involving approach of T-cell epitope prediction and their validation *in vivo/in vitro* is a very powerful strategy for rational antigen identification with the additional experimental work required to validate the epitope. This study involving extensive mining of the *C. jejuni* proteome for the identification of potential vaccine candidates can inspire similar strategies to be adopted for other species or pathogens for control of such serious disease. In recent times, the combined use of reverse vaccinology and immunoinformatics has emerged as the most promising approach in designing successful vaccine against infectious diseases as elucidated by these results. Further use of computational modeling and simulation shall predict the immunomodulatory effects of such vaccine candidates and thus offer an even better criterion for further *in vitro* or *in vivo* experimentation.

Acknowledgements The first author sincerely thanks the Institute of Engineering and Technology for providing necessary infrastructure and support for the work.

Compliance with Ethical Standards

Conflict of Interest Authors declare that they have no conflict of interest.

References

1. Van Vliet AH, Ketley JM (2001) Pathogenesis of enteric *Campylobacter* infection. *Symp Ser Soc Appl Microbiol* 30:45S–56S. doi:[10.1046/j.1365-2672.2001.01353.x](https://doi.org/10.1046/j.1365-2672.2001.01353.x)
2. Asakura M, Samosornsuk W, Taguchi M, Kobayashi K, Misawa N, Kusumoto M, Nishimura K, Matsuhisa A, Yamasaki S (2007) Comparative analysis of cytolethal distending toxin (cdt) genes among *Campylobacter jejuni*, *C. coli* and *C. fetus* strains. *Microb Pathog* 42:174–183. doi:[10.1016/j.micpath.2007.01.005](https://doi.org/10.1016/j.micpath.2007.01.005)
3. Dasti JI, Tareen AM, Lugert R, Zautner AE, Gross U (2010) *Campylobacter jejuni*: a brief overview on pathogenicity—associated factors and disease- mediating mechanisms. *Int J Med Microbiol* 300:205–211. doi:[10.1016/j.ijmm.2009.07.002](https://doi.org/10.1016/j.ijmm.2009.07.002)
4. Adedayo O, Kirkpatrick BD (2008) *Campylobacter jejuni* Infections: update on presentation, diagnosis, and management. *Hosp Phys* 44:9–15
5. Gould SR (1985) Toxic megacolon complicating *Campylobacter* colitis. *Br Med J (Clin Res Ed)* 291:1580. doi:[10.1136/bmj.291.6508.1580-e](https://doi.org/10.1136/bmj.291.6508.1580-e)
6. Butzler JP (1982) *Campylobacter* Enteritis. *Infection* 10:S67–69. doi:[10.1007/BF01640857](https://doi.org/10.1007/BF01640857)
7. Friedman CR, Neimann J, Wegener HC, Tauxe RV (2000) Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized Nations. In: Nachamkin I, Blaser MJ (eds) *Campylobacter*, 2nd edn. ASM International, Washington, pp 121–138
8. Gillespie IA, O'Brien SJ, Penman C, Tompkins D, Cowden J, Humphrey TJ (2008) Demographic determinants for *Campylobacter* infection in England and Wales: implications for future epidemiological studies. *Epidemiol Infect* 136:1717–1725. doi:[10.1017/S0950268808000319](https://doi.org/10.1017/S0950268808000319)

9. <https://www.cdc.gov/foodsafety/diseases/campylobacter/index.html>. Accessed 28 May 2017
10. Havelaar AH, Van Pelt W, Ang CW, Wagenaar JA, Van Putten JPM, Gross U, Newell DG (2009) Immunity to *Campylobacter*: its role in risk assessment and epidemiology. *Crit Rev Microbiol* 35:1–22. doi:10.1080/10408410802636017
11. Lin J (2009) Novel approaches for *Campylobacter* control in poultry. *Foodborne Pathog Dis* 6:755–765. doi:10.1089/fpd.2008.0247
12. Albert MJ (2014) Vaccines against *Campylobacter jejuni*. *Austin J Clin Immunol* 1:3
13. Rappuoli R (2000) Reverse vaccinology. *Curr Opin Microbiol* 3:445–450
14. Pizza M, Scarlato V, Masignani V et al (2000) Identification of vaccine candidates against serogroup B meningococcus by whole genome sequencing. *Science* 287:1816–1820. doi:10.1126/science.287.5459.1816
15. Gorringe AR, Pajon R (2012) Bexsero: a multicomponent vaccine for prevention of meningococcal disease. *Hum Vaccin Immunother* 8:174–183. doi:10.4161/hv.18500
16. Bagnoli F, Baudner B, Mishra RP, Bartolini E, Fiaschi L, Mariotti P, Nardi-Dei V, Boucher P, Rappuoli R (2011) Designing the next generation of vaccines for global public health. *OMICS* 15:545–566. doi:10.1089/omi.2010.0127
17. Moriel DG, Scarselli M, Serino L, Mora M, Rappuoli R, Masignani V (2009) Genome-based vaccine development: a short cut for the future. *Adv Exp Med Biol* 655:81–89. doi:10.1007/978-1-4419-1132-2_8
18. Burnens A, Stucki U, Nicolet J, Frey J (1995) Identification and characterization of an immunogenic outer membrane protein of *Campylobacter jejuni*. *J Clin Microbiol* 33:2826–2832
19. Petersen TN, Brunak S, Von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 8:785–786. doi:10.1038/nmeth.1701
20. Bendtsen JD, Kiemer L, Fausbøll A, Brunak S (2005) Non-classical protein secretion in bacteria. *BMC Microbiol* 5:58. doi:10.1186/1471-2180-5-58
21. Doytchinova IA, Flower DR (2007) VaxiJen : a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics* 8:4. doi:10.1186/1471-2105-8-4
22. Magnan CN, Zeller M, Kayala MA, Vigil A, Randall A, Felgner PL, Baldi P (2010) High-throughput prediction of protein antigenicity using protein microarray data. *Bioinformatics* 26:2936–2943. doi:10.1093/bioinformatics/btq551
23. Hirose S, Noguchi T (2013) ESPRESSO: a system for estimating protein expression. *Proteomics* 13:1444–1456. doi:10.1002/pmic.201200175
24. Krogh A, Larsson È, Heijne GV, Sonnhammer ELL (2001) Predicting transmembrane protein topology with a hidden markov model: application to complete genomes. *J Mol Biol* 305:567–580. doi:10.1006/jmbi.2000.4315
25. Moreno-hagelsieb G, Latimer K (2008) Choosing BLAST options for better detection of orthologs as reciprocal best hits. *Bioinformatics* 24:319–324. doi:10.1093/bioinformatics/btm585
26. Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O, Nielsen M (2007) Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC Bioinform* 8:424. doi:10.1186/1471-2105-8-424
27. Nielsen M, Lund O, NN-align (2009) An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. *BMC Bioinform* 10:296. doi:10.1186/1471-2105-10-296
28. Toussaint NC, Kohlbacher O (2009) OptiTope—a web server for the selection of an optimal set of peptides for epitope-based vaccines. *Nucleic Acids Res* 37:1–6. doi:10.1093/nar/gkp293
29. Liu I, Lo Y, Yang J (2011) PAComplex: a web server to infer peptide antigen families and binding models from TCR–pMHC complexes. *Nucleic Acids Res* 39:254–260. doi:10.1093/nar/gkr434
30. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW (2008) Evolutionarily conserved amino acids that control TCR-MHC interaction. *Annu Rev Immunol* 26:171–203. doi:10.1146/annurev.immunol.26.021607.090421
31. Beaver JE, Bourne PE, Ponomarenko JV (2007) EpitopeViewer: a Java application for the visualization and analysis of immune epitopes in the Immune Epitope Database and Analysis Resource (IEDB). *Immun Res* 3:1–6. doi:10.1186/1745-7580-3-3
32. Sette A, Vitiello A, Rehman B, Fowler P, Nayarsina R, Kast WM, Melief CJ, Oseroff C, Yuan L, Ruppert J (1994) The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J Immunol* 153:5586–5592. doi:10.1186/1471-2105-15-241