Proteome-wide screening for designing a multi-epitope vaccine against emerging pathogen *Campylobacter jejuni* using immunoinformatics approaches

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Master Of Science in Biotechnology

Department of Mathematics and Natural Sciences

Brac University

January 2025

Declaration:

It is hereby declared that:

1. The thesis submitted is my original work while completing a master's degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all of the main sources of help.

Student's Full Name & Signature:

_____

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Approval:

The thesis titled 'Proteome-wide screening for designing a multi-epitope vaccine against emerging pathogen *Campylobacter jejuni* (*C.jejuni)* using immunoinformatics approaches' submitted by Mariam Alsaied (23176018) of Spring 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology on the 30 of January 2025.

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Ethics Statement:

The thesis was conducted maintaining ethical standards at all stages of the research. No animals or human beings were harmed or involved in any aspect of this study. The computational methodologies employed ensured that no invasive or experimental procedures were required, thereby avoiding any potential ethical concerns related to biological testing. Furthermore, all data and tools used in the research were obtained from publicly accessible and reputable sources, ensuring compliance with data-sharing and intellectual property regulations. The study also maintained full adherence to biosafety and ecological protocols, ensuring that no harmful impact on the environment or public health occurred as a result of the research. These measures reflect the commitment to upholding integrity, responsibility, and respect for ethical principles throughout the research process.

Abstract:

Campylobacter jejuni (*C. jejuni)* is a leading cause of bacterial gastroenteritis worldwide, often resulting in severe complications such as Guillain-Barré syndrome and reactive arthritis. The rising prevalence of antibiotic-resistant strains underscores the urgent need for alternative preventive measures, such as vaccines. This study employs a comprehensive immunoinformatics approach to design a multi-epitope vaccine targeting *C. jejuni*. Out of the 1,800 proteins in the *C. jejuni* proteome, the major outer membrane protein (MOMP) was identified as the most antigenic candidate, with a VaxiJen score of 0.5059. MOMP was further analyzed to predict cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes, using tools such as NetCTL, IEDB, and VaxiJen. The selected epitopes were evaluated for immunogenicity, allergenicity, and toxicity, ensuring their suitability for vaccine development. Additionally, linear B-cell epitopes (LBL) were predicted to induce humoral immunity. The identified CTL and HTL epitopes were also analyzed for global population coverage, achieving significant inclusivity across diverse HLA alleles. The proposed vaccine construct integrates these epitopes to stimulate robust cellular and humoral immune responses, offering a promising candidate for further experimental validation. This study highlights the potential of computational methods in accelerating vaccine design and provides a framework for addressing the significant global burden of *C. jejuni* infections.

Keywords: *Campylobacter jejuni, C. jejuni,* vaccine, bacterial gastroenteritis, immunoinformatics, epitope, multi-epitope, CTL epitope, HTL epitope, LBL, MOMP, MOMP protein, immune responses, infections.

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Chapter 1
Introduction

The cases of enteric disease including bacterial gastroenteritis caused by *Campylobacter jejuni* and *Campylobacter coli* worldwide are 500 million cases annually (Kaakoush et al., 2015). However, although it has been the cause of most human infections, the development of a Campylobacter vaccine has not been achieved because of the unavailability of relevant animal models that can replicate the disease in humans (Young, 2007). Campylobacter species are among the main class of human bacterial pathogens contributing to millions of cases of bacterial gastroenteritis annually, and hence responsible for a considerable part of diarrhea morbidity worldwide. It spreads through the fecal-oral route in both high and low-income countries. A prospective study of over 22,000 children from seven different developing countries further emphasized this medical concern, because Campylobacter ranks in the top three pathogens that cause moderate to severe diarrhea in children aged between 24 to 59 months. The cost of illness attributable to Campylobacter-associated disease is high, with annual costs estimated at up to 5.6 billion dollars in the United States alone (Kotloff, K. L., 2013).

Campylobacter jejuni (C. jejuni) is the leading causative agent of campylobacteriosis which results in gastroenteritis due to diarrhea and associated causes including bacteria, bacteremia, and septicemia, and a few severe central nervous system disorders. This is due to the availability of modern-like treatment for children and infants as global foodborne sulphoses and are brought back by the elderly and travelers from not well-cooked poultry and meat. A successful vaccine is of great value in the management and prevention of *C.jejuni* colonization and *C. jejuni* infection in chickens and human. Continuous antigen presentation of small, non-pathogenic aspects of an infectious agent may be useful for prevention and therapy or could be derived or drug-phobic to avoid episodes of infection (Silva, J., 2011).

In terms of Campylobacter vaccine development, progress has been made as of 2024, though human use is still not available. Many varieties of Campylobacter including *Campylobacter jejuni* and *Campylobacter coli* are among the bacterial agents responsible for gastroenteritis and vaccination would be needed to reduce the impact of disease for instance in regions where the prevalence is high (WHO, 2024).

Due to the recognition of different vaccines as immunogenic epitopes or marginally effective immunogenic peptides, they are the most active parts of any antigenic subunit that undergoes immunological responses, either humoral or cellular. It can also determine and screen epitope-based vaccines completely by analyzing all possible MHC I-restricted and MHC II-restricted epitopes present in the whole proteome and that is the most promising approach (Kaakoush et al., 2015). The proteome-wide approaches towards the identification of B-cell epitopes are yet another useful and very effective mechanism in the field of immunoinformatics and a method in several successful candidate vaccine development for many pathogens. Thus, the primary objective of the present study is the targeting of more T-cell epitopes and B-cell epitopes of various proteins of *C. jejuni* for the formulation of suitable epitope-based multi-epitope vaccines.

Aim:

To target more T-cell epitopes and B-cell epitopes of various proteins of *C. jejuni* for the formulation of a suitable epitope-based multi-epitope vaccine.

Objectives:

- To reduce infections caused by *C. jejuni* in humans, especially infants, elderly, travelers, and immunocompromised patients, as well as animals.
- To control the reliance on antibiotics for the treatment of *C. jejuni* infections that have shown antibiotic resistance to fluoroquinolones, such as ciprofloxacin.
- To help with the economic burden and food safety in public health measurements in both low and high-income countries.
- To investigate the underlying mechanisms and effects of *Campylobacter jejuni* infection on impaired nutrient absorption within the gastrointestinal tract.

Chapter 2
Literature Review

The *Campylobacter jejuni (C. jejuni)* constituent is currently the most common cause of foodborne residual bacterial gastroenteritis in the world. The campylobacteriosis is caused by diarrhea and inflammation of the intestines, abdomen pain, feelings of nausea, shivering, and fever. Reactive arthritis, Guillain-Barre syndrome, or irritable bowel syndrome (IBS) develop in people after recovery from Campylobacteriosis. It was found that Farm rabbits infected with *C. jejuni* incorporated adjuvants in these models mutilated intestinal inflammation and focal inflammation was formed with ciliated epithelium gastroenteritis (Kaakoush et al., 2015). *C. jejuni* infection is the most common cause of zoonotic gastroenteritis with frequent chronic manifestations. Nevertheless, *C. jejuni*-induced pathologies are far from being understood. According to a novel study about *Campylobacter jejuni* impairs sodium transport and epithelial barrier function via cytokine release in human colon, finding suggested inhibition of epithelial sodium transport due to simultaneous decrease of b- and g-ENaC subunits. The Na+ absorption within the colonic epithelium is known to be compromised due to the acute confusion that is demonstrated about JNathatin measurements. Therefore, Na+ malabsorption was found as a mechanism of diarrhea. The sama encymphoma was also shown to disturb the function of ENaC e.g. 21 Of the IL-6 and IL-13, regulation of the level of ENaC activity would not only involve the transcriptional regulation of b and g subunits SD and G of ENaC.22 Such expression regulation has already been shown in other studies and applied to other factors such as MAP kinase activity or unrelated pathways (Kunzelmann et al., 2002; Mall et al., 2003).

It was, however, only very recently that the aforementioned reduction of both mRNA and protein levels of IL13 on ENaC in cell and mouse models has been comprehensively accounted for moving to the case of Campylobacter-induced ENaC disturbance, this was irrespective of treatment in that there are already bacteria directly disrupting ENaC function in vitro, and symptoms that enhance this activity are the cytokines IFNg, TNFa, IL-13, and IL-1b that occur following the early stage of the infection and are released by LPL after the bacteria penetrate more deeply into the mucosa (cryptitis) (Heitzmann & Warth, 2008; Kunzelmann et al., 2002). Similar outcomes were also seen from mice models of salmonella infection ENaC dysregulation and CFTR and DRA downregulation led to decreased gut absorption which resulted in diarrhea.

Campylobacter jejuni is one of the leading causes of bacterial gastroenteritis worldwide, significantly impacting public health, especially in low- and middle-income countries. This Gram-negative, spiral-shaped bacterium is predominantly transmitted through contaminated food, water, and undercooked poultry. Infection with *C. jejuni* often results in symptoms ranging from diarrhea and abdominal cramping to severe outcomes such as Guillain-Barré syndrome (GBS) and reactive arthritis, underscoring its potential to cause long-term complications. The global burden of *C. jejuni* infections highlights the urgent need for effective prevention strategies. Despite advancements in hygiene and food safety, infections remain widespread, partly due to the bacterium's ability to evade the host immune system through antigenic variation and molecular mimicry. The rising prevalence of antibiotic-resistant *C. jejuni* strains further complicates treatment, making vaccine development a critical priority. Peptide-based vaccines have emerged as a promising approach, offering safety, specificity, and the ability to target conserved antigenic regions of the pathogen. By leveraging computational tools, this study aims to identify immunogenic epitopes from the *C.jejuni* proteome to design a multi-epitope vaccine construct capable of eliciting robust cellular and humoral immune responses.

Campylobacter jejuni infections account for an estimated 96 million cases of diarrhea annually worldwide (Kaakoush et al., 2015). The bacterium primarily colonizes the intestinal epithelium, where it adheres to and invades host cells using virulent factors such as CadF, FlaA, and JlpA. The lipooligosaccharides (LOS) on its surface can mimic host gangliosides, triggering autoimmune responses like Guillain-Barré syndrome (GBS) in some individuals (Yuki, 2001). These factors, combined with its genetic adaptability and antigenic diversity, enable *C.jejuni* to evade host immune defenses and establish infection.

While antibiotic therapy, particularly with macrolides and fluoroquinolones, remains the mainstay for treating severe *C.jejuni* infections, the emergence of multidrug-resistant (MDR) strains poses a growing threat (Luangtongkum et al., 2009). The reliance on antibiotics has also raised concerns about the development of resistance in zoonotic reservoirs, further emphasizing the need for alternative preventive measures such as vaccines. Efforts to develop a vaccine against *C. jejuni* have largely focused on targeting conserved antigens and immunogenic epitopes. Proteins such as the major outer membrane protein (MOMP), cadF, and flagellar components are recognized for their roles in pathogenesis and immune recognition (Riddle et al.,

2016). Recent studies leveraging immunoinformatics tools have demonstrated the potential of epitope-based vaccines, which target T-cell and B-cell epitopes to elicit a comprehensive immune response (Doytchinova & Flower, 2007). Immunoinformatics tools have revolutionized vaccine development by enabling rapid and accurate identification of epitopes that interact with MHC molecules and stimulate immune responses. Tools such as VaxiJen, NetCTL, and IEDB MHC binding prediction are widely used to predict antigenicity, immunogenicity, and population coverage (Calis et al., 2013; Dhanda et al., 2013). These in silico approaches significantly reduce the time and cost of vaccine development by narrowing down potential candidates for experimental validation. While several immunogenic proteins of *C. jejuni* have been studied, there remains a need to systematically analyze its proteome to identify epitopes capable of eliciting strong and specific immune responses. This study seeks to fill this gap by using a comprehensive computational pipeline to design a multi-epitope vaccine targeting both cellular and humoral immunity, to address the global burden of *C. jejuni* infections.

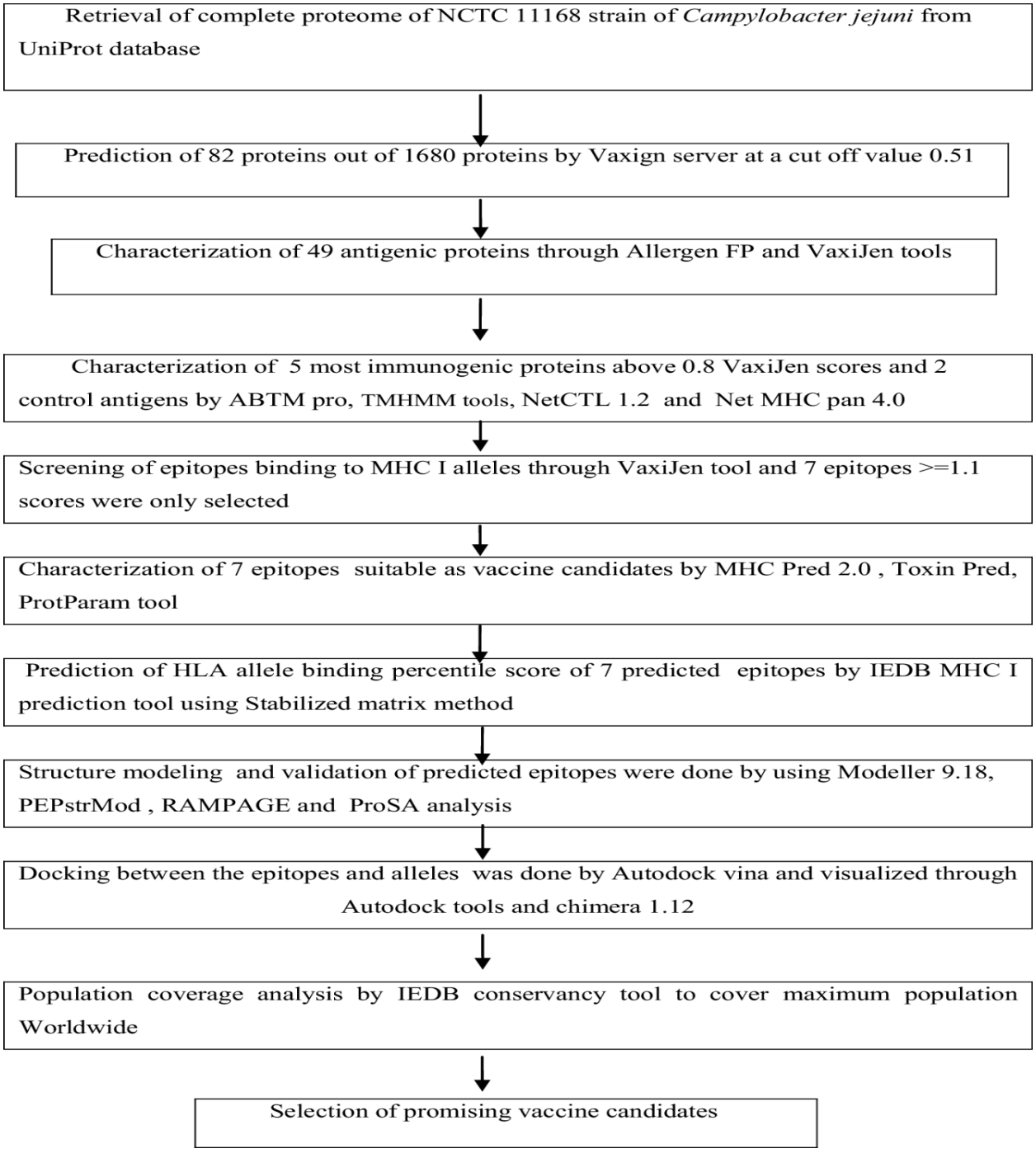


Figure 1: Flowchart portraying the screening of potential vaccine candidates from the *Campylobacter jejuni* genome.

The infection of *Campylobacter jejuni* and its interaction with T-cells and B-cells involves multiple mechanisms aimed at immune evasion, inflammation, and sometimes autoimmunity.

C. jejuni infects the host primarily through contaminated food and water, colonizing the intestinal epithelium. The bacteria attach to epithelial cells using adhesins (e.g., CadF and JlpA proteins) and then invade these cells to evade immune detection (Konkel et al., 2001; Backert et al., 2018). The immune system recognizes *C. jejuni* via pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) on macrophages and dendritic cells. These receptors detect bacterial components like lipopolysaccharides (LPS), flagellin, and lipooligosaccharides (LOS), initiating an immune response. PRR activation triggers the release of cytokines and chemokines (e.g., IL-8, TNF-α, IL-1β), leading to inflammation and recruitment of immune cells, including T-cells and B-cells, to the infection site. Antigen-presenting cells (APCs) such as dendritic cells engulf *C. jejuni* or its antigens and migrate to lymphoid tissues, where they present antigens to T-cells, initiating adaptive immunity. APCs process *C. jejuni* antigens and present them on MHC class II molecules to CD4+ T-helper (Th) cells and on MHC class I molecules to CD8+ cytotoxic T-cells (CTLs). In response to *C. jejuni*, CD4+ T-cells often differentiate into Th1 and Th17 cells. Th1 cells produce IFN-γ and TNF-α, which activate macrophages and enhance the killing of intracellular pathogens (Jones et al., 2021). Th17 cells release IL-17, contributing to neutrophil recruitment and inflammation, which helps clear the bacteria but can also cause tissue damage. CD8+ Cytotoxic T-cells recognize *C. jejuni* antigens presented on MHC I molecules by infected or damaged cells, leading to the killing of these cells to control bacterial spread. B-Cell Activation as activated T-helper cells, especially Th1 cells help B-cells recognize *C. jejuni* antigens, leading to B-cell activation and differentiation into plasma cells. Plasma cells produce antibodies (IgA, IgM, and IgG) targeting *C. jejuni* antigens. IgA antibodies are particularly important in mucosal immunity and are secreted in the gut, where they neutralize the bacteria and prevent further adherence and invasion. IgG and IgM antibodies in the bloodstream can help neutralize bacteria that translocate across the epithelium (van Spreeuwel et al., 1985; Walker et al., 2018). *C. jejuni* can alter surface proteins (e.g., LOS and flagellin) to stop immune recognition and antibody targeting. The lipooligosaccharide (LOS) structure of *C. jejuni* can mimic human gangliosides. This molecular mimicry can lead to autoimmunity by inducing antibodies that cross-react with human nerve tissues, potentially triggering Guillain-Barré syndrome (GBS) and reactive arthritis. *C. jejuni* can stimulate regulatory T-cells (Tregs), which

produce IL-10 and other immunosuppressive cytokines to dampen immune responses and inflammation. This modulation may allow the bacteria to persist in the gut longer by avoiding excessive immune clearance. After infection is cleared, some T-cells and B-cells remain as memory cells, allowing the immune system to respond more rapidly upon reexposure to *C. jejuni* (Smith et al., 2020). However, natural immunity to *C. jejuni* is generally not long-lasting, and individuals may be susceptible to reinfection, driving the need for a potential vaccine.

Chapter 3
Genomic analysis & Impaired Nutrient Absorption

3.1. Genomic Analysis of *C. jejuni*

The genome of *Campylobacter jejuni* (*C. jejuni)* has been extensively studied to understand its pathogenicity, virulence, and adaptability to various hosts and environmental conditions. The sequencing of its genome, particularly strain NCTC 11168, has provided significant insights into its molecular mechanisms and survival strategies.

The genome of *C. jejuni* is relatively small, approximately 1.6-1.8 Mb, and circular. It encodes around 1,600 to 1,800 proteins, with a GC content of 30-31%, reflecting its adaptation to a microaerophilic lifestyle. The genome lacks several classical regulatory systems (e.g., two-component regulatory systems), highlighting its reliance on post-transcriptional regulation for gene expression. Virulence-Associated Genes including CadF and JlpA: Adhesins involved in binding to fibronectin on host epithelial cells. FlaA and FlaB: Flagellar proteins critical for motility and colonization. Cia proteins: Campylobacter invasion antigens that facilitate epithelial cell invasion. LOS and Capsule Biosynthesis Genes: Responsible for immune evasion and molecular mimicry, contributing to complications like Guillain-Barré Syndrome (GBS). *C. jejuni* exhibits high genomic plasticity, facilitated by horizontal gene transfer, phase variation, and recombination. These mechanisms allow it to adapt to diverse environments and hosts, including changes in nutrient availability.

The genomic architecture of *Campylobacter jejuni* plays a pivotal role in its ability to colonize the gastrointestinal tract, evade host defenses, and impair nutrient absorption. Genomic studies reveal that *C. jejuni* possesses a relatively small genome (~1.6–1.7 Mbp) with high genetic plasticity, enabling rapid adaptation to various environmental and host conditions (Parkhill et al., 2000). Key genes involved in motility (flaA, flaB), adhesion (cadF, pEB1), and immune evasion (LOS biosynthesis genes) facilitate its pathogenicity and persistence within the intestinal epithelium. Furthermore, *C. jejuni* exploits its genomic machinery to alter host cell signaling

pathways, disrupting the structural integrity of intestinal tight junctions and leading to increased intestinal permeability (Friis et al., 2005).

3.2. Impaired Nutrient Absorption Due to *C. jejuni*

Infection with *Campylobacter jejuni* is associated with significant disruptions in the host's intestinal function, particularly affecting nutrient absorption. The bacterium's interaction with the intestinal epithelium triggers inflammatory responses and structural damage that compromise the gut's absorptive capacity. Mechanisms of Impairment includes damage to Intestinal Epithelium. *C. jejuni* adheres to and invades intestinal epithelial cells, inducing apoptosis and disrupting tight junctions. This results in increased intestinal permeability ("leaky gut") and reduced surface area for absorption. Inflammation and Cytokine Release as infection triggers the release of pro-inflammatory cytokines like IL-8, TNF-α, and IFN-γ, leading to chronic inflammation. This inflammatory milieu exacerbates epithelial damage and reduces the efficiency of nutrient transporters. Disruption of Microvilli: the structural integrity of microvilli on enterocytes is compromised, reducing the surface area available for nutrient absorption. The impact on nutrient absorption: Carbohydrates decreased activity of disaccharidases (e.g., lactase) due to epithelial damage leads to malabsorption of sugars, often resulting in osmotic diarrhea. Proteins: damage to peptide transporters reduces amino acid uptake, impairing protein nutrition. Lipids: inflammation-induced bile acid malabsorption affects lipid digestion and absorption, contributing to steatorrhea (fatty stools). Micronutrients: Deficiencies in iron, calcium, zinc, and vitamins (A, D, E, K) are commonly observed due to impaired transporter function and malabsorption. The consequences of malabsorption can include acute malnutrition as persistent diarrhea and nutrient loss can lead to weight loss and acute malnutrition, particularly in children. Growth retardation chronic infections in children are linked to stunted growth and developmental delays, often referred to as environmental enteropathy. Immune dysfunction and nutrient deficiencies weaken the immune system, creating a vicious cycle of infection and malnutrition. The role of genomic factors in nutrient absorption impairment, virulence genes, and enterocyte damage genes encoding for CadF, FlaA, and Cia proteins facilitate adhesion and invasion, directly contributing to epithelial barrier disruption. LOS biosynthesis genes mimic host glycosylation patterns, allowing the bacteria to evade immune detection while triggering autoimmune-like responses that worsen gut damage. Regulation of Host-Microbiota interactions as the *C. jejuni* disrupts the

gut microbiota, favoring the overgrowth of pathogenic bacteria and reducing beneficial microbial populations. This dysbiosis negatively affects short-chain fatty acid (SCFA) production, which is essential for gut health and nutrient absorption. The genomic basis of *C. jejuni* pathogenesis and its role in nutrient malabsorption opens avenues for developing targeted therapies, such as multi-epitope vaccines targeting adhesion and invasion proteins (e.g., CadF, JlpA) to prevent epithelial damage. Also, probiotics and microbiota restoration use probiotics to restore gut microbiota balance and improve nutrient absorption. Therapeutics development of small molecules or biologics to block key virulence factors and reduce epithelial damage. The genomic analysis of *Campylobacter jejuni* highlights its sophisticated virulence mechanisms that enable host colonization, immune evasion, and disruption of intestinal nutrient absorption. Addressing the dual challenges of bacterial pathogenesis and malnutrition requires a multidisciplinary approach, combining genomic insights with novel therapeutic and preventive strategies.

Chapter 4

Methodology

4.1 Retrieval of proteome and prediction of the highest antigenic protein:

The FASTA format sequence file was completed to retrieve the whole *Campylobacter jejuni (C. jejuni)* proteome which was downloaded from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC https://www.bv-brc.org/). It derives from the National Center for Biotechnology Information (NCBI) databases and assembles free draft and complete genomes from all three domains of life. (Markowitz et al., 2012) The dynamic tool Phylogenetic Profiler available at IMG/M system allows for the extraction of support protein sequences for any or each gene in the relation despite available yeast protein homologues from other genomes. Antigenicity as a key consideration is defined as the capacity to elicit any form of an immune response against the antigen, therefore, peptide-based vaccines should consider proteins with high antigenicity. Therefore, all provided proteins from the proteome of *C. jejuni* were scanned through the VaxiJen 2.0 server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) at a 0.5 threshold. It has a range of 70-89% accuracy in varying levels of scope whereby the use of auto cross-covariance ACC transformation method enhances more (Doytchinova & Flower, 2007).

4.2. Prediction of CTL epitopes and their MHC-I binding alleles:

Cytotoxic T-cells, the killers of the immune system, express T-cell receptors (TCRs) that can detect a specific antigen (Chaudhri et al., 2009). Thus, the prediction of CTL epitopes is also critical to rational vaccine design. Results were submitted to the NetCTL 1.2 server (https://services.healthtech.dtu.dk/services/NetCTL-1.2/) for the prediction of CTL epitopes with a 0.5 threshold (Larsen et al., 2007). There are three criteria that this server defines the prediction for CTL epitopes where MHC-I binding, C-terminal cleavage, and TAP transport efficiency. An artificial neural network was applied to predict MHC-I binding and C-terminal cleavage and the weight matrix method was used to determine the efficiency of TAP transporter (Larsen et al. MHC-I binding alleles for each CTL epitope were also predicted using the consensus method in the IEDB analysis tool (Moutaftsi et al., 2006). The percentile rank 2 was considered as the threshold value while Human was selected as the MHC source species.

4.3. Assessment of CTL epitopes for immunogenicity, allergenicity and toxicity:

The immunogenic potential of CTL epitopes are the epitome of the competence of vaccine efficaciousness (Adhikari & Rahman, 2017). Hence, initially expected CTL epitopes have been screened for immunogenicity by way of MHC-I Immunogenicity device of the IEDB server (http://tools.immuneepitope.org/mhci/). Furthermore, antigenicity was predicted using VaxiJen 2.0 server (Doytchinova & Flower, 2007) to investigate their immunogenic potential. Free from allergenic potential in vaccine components Therefore the allergenicity prediction was carried out using AllerTOP 2.0 server which predicts by j-nearest neighbors' method with 88.7% accuracy (Dimitrov, Bangov, Flower & Doytchinova, 2014). Additionally, toxic epitopes must be removed as these could derail the goal of vaccine development. The ToxinPred server was used to screen the toxic CTL epitope. This server works based on the different properties of peptides using machine learning techniques and quantitative matrix (Gupta et al., 2013).

4.4. Prediction of HTL epitopes and their MHC-II binding alleles:

Helper T-cells, integral components of the adaptive immune system, play a crucial role in eliciting both cellular and humoral immune responses to foreign antigens. Consequently, Helper T-cell lymphocyte (HTL) epitopes that interact with MHC class II alleles are pivotal for the strategic development of vaccines (Nielsen, Lundegaard, & Lund, 2007). In this study, the HTL epitopes and their associated MHC-II alleles were predicted using the IEDB (http://tools.iedb.org/mhciinp/) MHC II binding tool via the consensus method (Wang et al., 2010). This method generates a percentile rank by comparing the peptide's IC50 to those of random peptides from the SWISS-PROT database (Paul, Sidney, Sette, & Peters, 2016). A lower percentile rank indicates stronger binding affinity, with a threshold of percentile rank 2 denoting high affinity.

4.5. Identification of cytokine-inducing HTL epitopes:

Helper T-cells play a vital role in activating cytotoxic T-cells and other immune cells by releasing various cytokines, such as interferon-gamma (IFN-γ), interleukin-4 (IL-4), and interleukin-10 (IL-10) (Luckheeram et al., 2012). Consequently, HTL epitopes that induce cytokine production are essential for the development of vaccines and immunotherapies. To identify HTL epitopes that produce IFN-γ, we utilized the IFNepitope server (https://webs.iiitd.edu.in/raghava/ifnepitope/predict.php), employing a hybrid approach that combines motif analysis and support vector machine (SVM) techniques, using the IFN-γ versus Non-IFN-γ model as the default setting (Dhanda et al., 2013). Additionally, the properties of IL-4 and IL-10 inducing epitopes were predicted using the IL4pred (Dhanda et al., 2013) and IL10 pred (https://webs.iiitd.edu.in/raghava/il4pred/design.php) (Nagpal et al., 2017) servers, respectively. These tools are specifically designed for the in silico prediction of peptides that induce interleukins, with IL4pred and IL10pred utilizing SVM and hybrid methods, respectively, while maintaining default parameters.

4.6. Prediction and assessment of LBL epitopes:

B-cells are responsible for humoral immunity by producing immunoglobulins that can neutralize antigens upon binding (Cooper, 2015). There are two types of B-cell epitopes: linear (continuous) and conformational (discontinuous). The ElliPro tool (http://tools.iedb.org/ellipro/download/) from the IEDB database is capable of predicting both types of B-cell epitopes based on their flexibility and solvent accessibility (Ponomarenko et al., 2008). However, for vaccine design purposes, only linear epitopes are taken into account. Consequently, we focused on predicting linear B-cell (LBL) epitopes using the ElliPro tool with its default settings. This tool employs three distinct algorithms: one for approximating protein shape, another for calculating the protrusion index (PI) of residues, and a third for clustering neighboring residues. Each predicted epitope is assigned a PI score, with higher PI values indicating greater solvent accessibility (Ponomarenko et al., 2008). The identified LBL epitopes were then further assessed for their antigenic, allergenic, and toxic properties using the VaxiJen 2.0, AllerTOP 2.0, and ToxinPred servers, respectively.

Chapter 5

Results & Data Analysis

Out of total of 1,800 proteins in *C. jejuni* proteome in its genome were evaluated with the VaxiJen 2.0 server that predicts antigenic potency by assigning each protein a score. The higher the score, the higher the antigenicity. The highest score of the first two probable periplasmic proteins. Overall Prediction for the Protective Antigen = 0.5059 (Probable Antigene). The major outer membrane protein (MOMP) of *Campylobacter jejuni* has 407 amino acids. Each line of the sequence appears to be approximately 50 amino acids, with eight full lines and an additional seven amino acids in the final line. Due to the highest antigenicity and possible association with bacterial virulence, the MOMP-related protein of *C.jejuni* was selected for further analyses in the designing of a multi-epitope vaccine construct. The finally selected CTL and HTL epitopes and their corresponding HLA alleles were used to estimate the population coverage both individually and in combination.

5.1: Antigenicity Prediction:

Antigenicity prediction was performed using Vaxijen 2.0 server. The protein sequence corresponds to the major outer membrane protein (MOMP) of *Campylobacter jejuni*. MOMP is one of the most abundant and immunogenic proteins in *C. jejuni* and is widely studied as a vaccine target due to its role in bacterial structure, permeability, and immune recognition. Due to its surface exposure and conservation among *C. jejuni* strains, MOMP is recognized by the immune system, making it a prime candidate for vaccine development. MOMP is considered a promising target for *C. jejuni* vaccine research, as antibodies against this protein could potentially neutralize the pathogen and prevent infection. At a threshold of 0.5 with bacteria as the target organism. The GI number, protein name, and FASTA sequence along with their antigenicity values are tabulated below:

MREALTSGKFLKLAGMMLAAAALLLTSLAAA

TAQERPRTLFDLLFGSRQAQPQRQYERPAPQ

RVKPKPKRPRASSPAARAAAPSAAAPAVEIA

TKKPDAKKILVVGDFIGNGLAEGLDAAFATD

PDLTVATRVNGSSGFVRDDYFNWPDNIGKIL

DEEKPAAVVVMIGANDRQAITANGNSLQPRS

PEWNAEYQKRVAAFTKVISDRHYPLVWVGQP

PFRPKGMSQDMLALNEIYRNAAEKAGGKFAD

VWDGFVDEEGNFTQTGFDINGQTARLRANDG

INITSTGKRKLAFYAEKPLHAYLGVSREEGN

SLPATHSLDPSKPVDRVAPVSLRDINKDDSG

ILLGGTPAMQAKPLKTEQKKANPSPGRADDF

SWPRNGKAPRK

Figure 2: FASTA sequence for *C. jejuni* of Overall Prediction for the Protective Antigen = 0.5059 (Probable ANTIGEN).

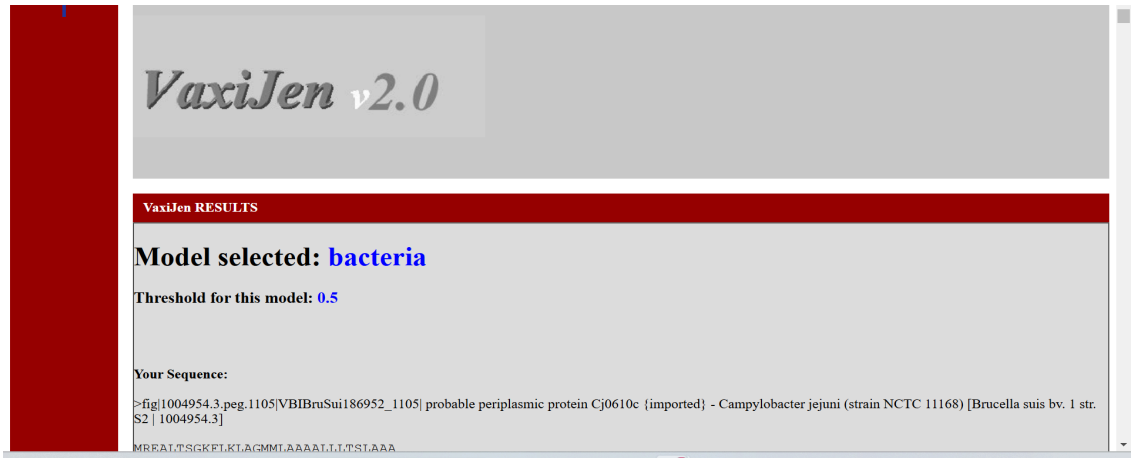
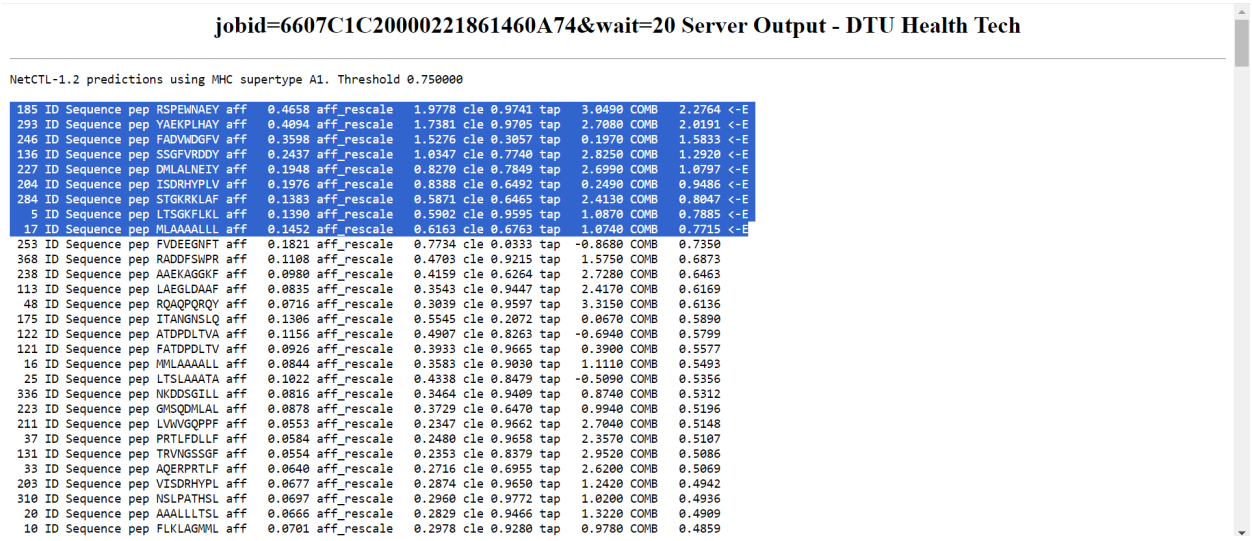


Figure 3 : Results from VaxiJen 2.0 of Bacteria at Threshold: 0.5.

5.2 CTL Epitope Identification:

The CTL epitopes are essential for the generation of enhanced immune response within the host system. They are highly ag-specific and express TCRs that can fight the particular ag.

Epitopes were identified using the Net CTL 1.2 server. A Threshold Level of 0.75, epitopes were identified of the A1 super type of MHC I alleles. The underlying algorithm used in this case is built upon the principle of neural networks. The criterion of combined score is the major determinant of epitope selection. This combined score is assigned to the corresponding epitopes based on parameters of C terminal cleavage & TAP transport efficiency. A minimal Threshold of 0.15 & 0.05 were respectively set in this regard. The final data are tabulated below:



jobid=6607C1C20000221861460A74&wait=20 Server Output - DTU Health Tech

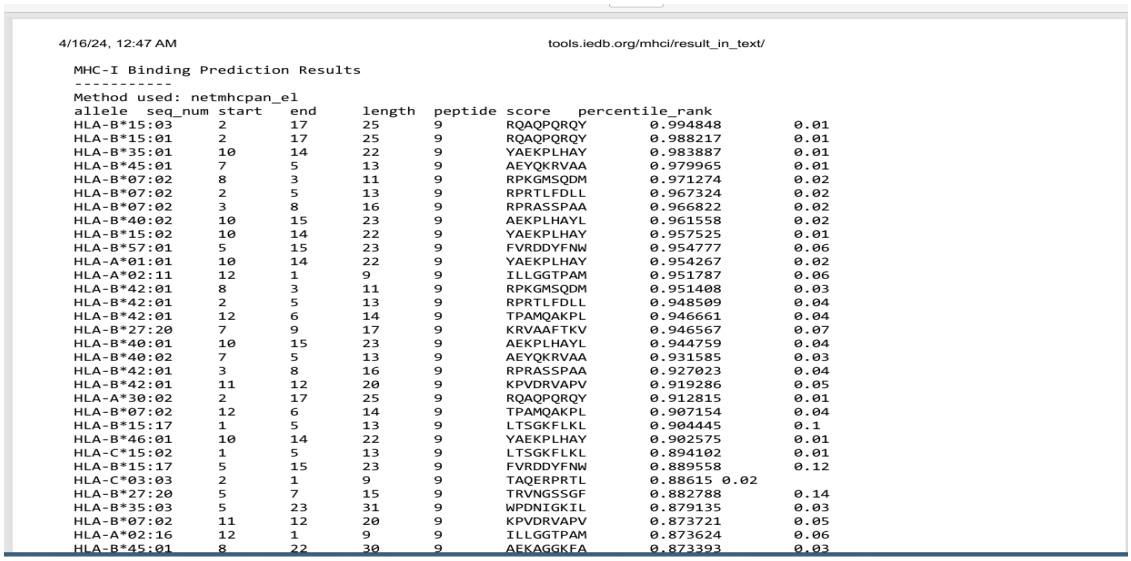
NetCTL-1.2 predictions using MHC supertype A1. Threshold 0.750000

185	ID	Sequence	pep	RSPEWNAEY	aff	0.4658	aff_rescale	1.9778	cle	0.9741	tap	3.0490	COMB	2.2764 <-E
293	ID	Sequence	pep	YAEKPLHAY	aff	0.4094	aff_rescale	1.7381	cle	0.9705	tap	2.7080	COMB	2.0191 <-E
246	ID	Sequence	pep	FADVWDGFV	aff	0.3598	aff_rescale	1.5276	cle	0.3057	tap	0.1970	COMB	1.5833 <-E
136	ID	Sequence	pep	SSGFVRDDY	aff	0.2437	aff_rescale	1.0347	cle	0.7740	tap	2.8250	COMB	1.2920 <-E
227	ID	Sequence	pep	DMLALNEIY	aff	0.1948	aff_rescale	0.8270	cle	0.7849	tap	2.6990	COMB	1.0797 <-E
204	ID	Sequence	pep	ISDRHYPLV	aff	0.1976	aff_rescale	0.8388	cle	0.6492	tap	0.2490	COMB	0.9486 <-E
284	ID	Sequence	pep	STGKRKLAF	aff	0.1383	aff_rescale	0.5871	cle	0.6465	tap	2.4130	COMB	0.8047 <-E
5	ID	Sequence	pep	LTSGKFLKL	aff	0.1390	aff_rescale	0.5902	cle	0.9595	tap	1.0870	COMB	0.7885 <-E
17	ID	Sequence	pep	MLAAAALLL	aff	0.1452	aff_rescale	0.6163	cle	0.6763	tap	1.0740	COMB	0.7715 <-E
253	ID	Sequence	pep	FVDEEGNFT	aff	0.1821	aff_rescale	0.7734	cle	0.0333	tap	-0.8680	COMB	0.7350
368	ID	Sequence	pep	RADDFSWPR	aff	0.1108	aff_rescale	0.4703	cle	0.9215	tap	1.5750	COMB	0.6873
238	ID	Sequence	pep	AAEKAGGKF	aff	0.0980	aff_rescale	0.4159	cle	0.6264	tap	2.7280	COMB	0.6463
113	ID	Sequence	pep	LAEGLDAAF	aff	0.0835	aff_rescale	0.3543	cle	0.9447	tap	2.4170	COMB	0.6169
48	ID	Sequence	pep	RQAQPQRQY	aff	0.0716	aff_rescale	0.3039	cle	0.9597	tap	3.3150	COMB	0.6136
175	ID	Sequence	pep	ITANGNSLQ	aff	0.1306	aff_rescale	0.5545	cle	0.2072	tap	0.0670	COMB	0.5890
122	ID	Sequence	pep	ATDPDLTVA	aff	0.1156	aff_rescale	0.4907	cle	0.8263	tap	-0.6940	COMB	0.5799
121	ID	Sequence	pep	FATDPDLTV	aff	0.0926	aff_rescale	0.3933	cle	0.9665	tap	0.3900	COMB	0.5577
16	ID	Sequence	pep	MMLAAAALL	aff	0.0844	aff_rescale	0.3583	cle	0.9030	tap	1.1110	COMB	0.5493
25	ID	Sequence	pep	LTSLAAATA	aff	0.1022	aff_rescale	0.4338	cle	0.8479	tap	-0.5090	COMB	0.5356
336	ID	Sequence	pep	NKDDSGILL	aff	0.0816	aff_rescale	0.3464	cle	0.9409	tap	0.8740	COMB	0.5312
223	ID	Sequence	pep	GMSQDMLAL	aff	0.0878	aff_rescale	0.3729	cle	0.6470	tap	0.9940	COMB	0.5196
211	ID	Sequence	pep	LVWVGQPPF	aff	0.0553	aff_rescale	0.2347	cle	0.9662	tap	2.7040	COMB	0.5148
37	ID	Sequence	pep	PRTLFDLLF	aff	0.0584	aff_rescale	0.2480	cle	0.9658	tap	2.3570	COMB	0.5107
131	ID	Sequence	pep	TRVNGSSGF	aff	0.0554	aff_rescale	0.2353	cle	0.8379	tap	2.9520	COMB	0.5086
33	ID	Sequence	pep	AQERPRTLF	aff	0.0640	aff_rescale	0.2716	cle	0.6955	tap	2.6200	COMB	0.5069
203	ID	Sequence	pep	VISDRHYPL	aff	0.0677	aff_rescale	0.2874	cle	0.9650	tap	1.2420	COMB	0.4942
310	ID	Sequence	pep	NSLPATHSL	aff	0.0697	aff_rescale	0.2960	cle	0.9772	tap	1.0200	COMB	0.4936
20	ID	Sequence	pep	AAALLLTSL	aff	0.0666	aff_rescale	0.2829	cle	0.9466	tap	1.3220	COMB	0.4909
10	ID	Sequence	pep	FLKLAGMML	aff	0.0701	aff_rescale	0.2978	cle	0.9280	tap	0.9780	COMB	0.4859

Figure 4: Results from CTL epitopes prediction of Net CTL 1.2 server.

5.3 MHC I allele specificity of CTL Epitope:

Epitopes that were identified in section 4.2 were used as input in this step to obtain MHC I alleles using the IEDB server assigned for this purpose. The 2009-09-01 B version was used for this purpose. The parameter used in this regard of epitope selection is percentile rank. A lower percentile rank implies a higher binding affinity and vice versa. A choice of minimal threshold of ≤2 for epitope selection in this regard. Allele-specific binding of CTL epitope and MHC I alleles with corresponding binding affinity in terms of percentile rank.



4/16/24, 12:47 AM tools.iedb.org/mhci/result_in_text/

MHC-I Binding Prediction Results

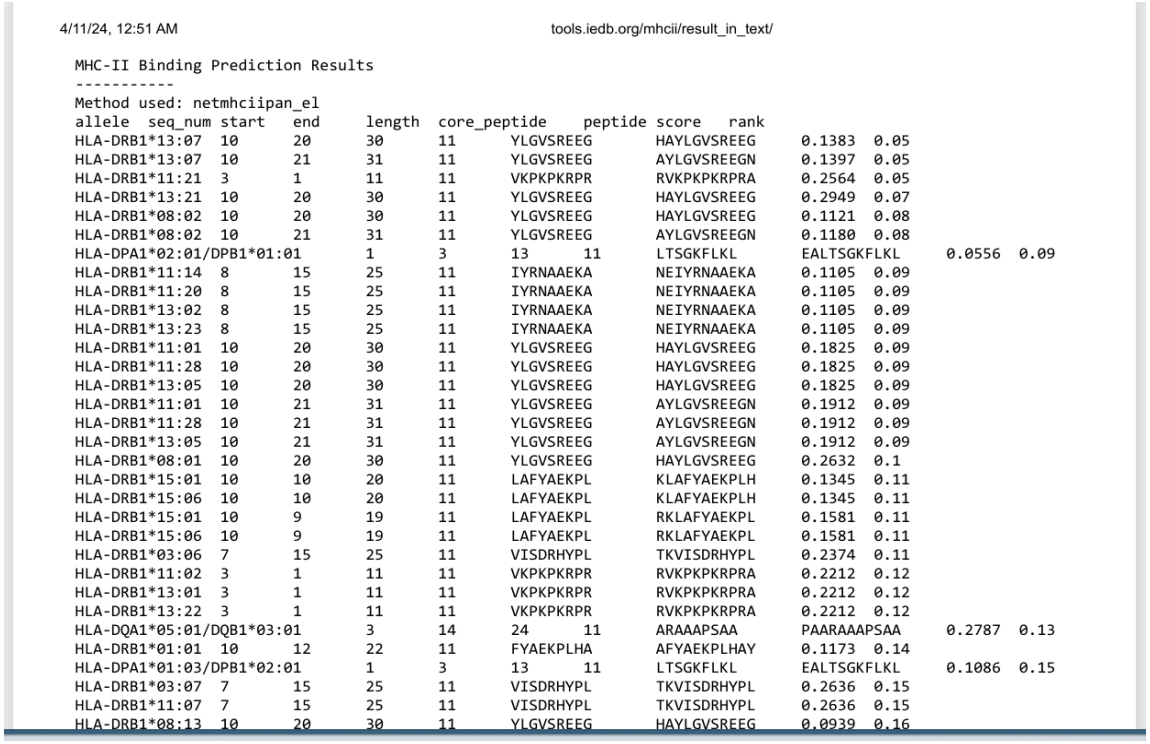
Method used: netmhcpan_el

allele	seq_num	start	end	length	peptide	score	percentile_rank
HLA-B*15:03	2	17	25	9	RQAQPQRQY	0.994848	0.01
HLA-B*15:01	2	17	25	9	RQAQPQRQY	0.988217	0.01
HLA-B*35:01	10	14	22	9	YAEKPLHAY	0.983887	0.01
HLA-B*45:01	7	5	13	9	AEYQKRVAA	0.979965	0.01
HLA-B*07:02	8	3	11	9	RPKGMSQDM	0.971274	0.02
HLA-B*07:02	2	5	13	9	RPRTLFDLL	0.967324	0.02
HLA-B*07:02	3	8	16	9	RPRASSPAA	0.966822	0.02
HLA-B*40:02	10	15	23	9	AEKPLHAYL	0.961558	0.02
HLA-B*15:02	10	14	22	9	YAEKPLHAY	0.957525	0.01
HLA-B*57:01	5	15	23	9	FVRDDYFNW	0.954777	0.06
HLA-A*01:01	10	14	22	9	YAEKPLHAY	0.954267	0.02
HLA-A*02:11	12	1	9	9	ILLGGTPAM	0.951787	0.06
HLA-B*42:01	8	3	11	9	RPKGMSQDM	0.951408	0.03
HLA-B*42:01	2	5	13	9	RPRTLFDLL	0.948509	0.04
HLA-B*42:01	12	6	14	9	TPAMQAKPL	0.946661	0.04
HLA-B*27:20	7	9	17	9	KRVAAFTKV	0.946567	0.07
HLA-B*40:01	10	15	23	9	AEKPLHAYL	0.944759	0.04
HLA-B*40:02	7	5	13	9	AEYQKRVAA	0.931585	0.03
HLA-B*42:01	3	8	16	9	RPRASSPAA	0.927023	0.04
HLA-B*42:01	11	12	20	9	KPVDRVAPV	0.919286	0.05
HLA-A*30:02	2	17	25	9	RQAQPQRQY	0.912815	0.01
HLA-B*07:02	12	6	14	9	TPAMQAKPL	0.907154	0.04
HLA-B*15:17	1	5	13	9	LTSGKFLKL	0.904445	0.1
HLA-B*46:01	10	14	22	9	YAEKPLHAY	0.902575	0.01
HLA-C*15:02	1	5	13	9	LTSGKFLKL	0.894102	0.01
HLA-B*15:17	5	15	23	9	FVRDDYFNW	0.889558	0.12
HLA-C*03:03	2	1	9	9	TAQERPRTL	0.88615	0.02
HLA-B*27:20	5	7	15	9	TRVNGSSGF	0.882788	0.14
HLA-B*35:03	5	23	31	9	WPDNIGKIL	0.879135	0.03
HLA-B*07:02	11	12	20	9	KPVDRVAPV	0.873721	0.05
HLA-A*02:16	12	1	9	9	ILLGGTPAM	0.873624	0.06
HLA-B*45:01	8	22	30	9	AEKAGGKFA	0.873393	0.03

Figure 5: MHC I Binding prediction results in iedb.

5.4 MHC II allele identification for HTL epitopes:

MHC II alleles are essential players in the enhancement of the Fadaptive immunity of a host, since they do so by binding to HTL and thus generate humoral & cell mediated immunity. MHC II alleles are identified using an IEDB server as mentioned earlier. MHC II alleles were identified using the primary antigen as an input. The indicator for MHC II allele identification is also percentile rank; a lower percentile rank indicates a higher binding affinity and vice versa. However, in this case a percentile rank of ≤ 0.5 was used for allele identification.



4/11/24, 12:51 AM tools.iedb.org/mhcii/result_in_text/

MHC-II Binding Prediction Results

Method used: netmhciipan_el

allele	seq_num	start	end	length	core_peptide	peptide	score	rank		
HLA-DRB1*13:07	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.1383	0.05		
HLA-DRB1*13:07	10	21	31	11	YLGVSREEG	AYLGVSREEGN	0.1397	0.05		
HLA-DRB1*11:21	3	1	11	11	VKPKPKRPR	RVKPKPKRPRA	0.2564	0.05		
HLA-DRB1*13:21	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.2949	0.07		
HLA-DRB1*08:02	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.1121	0.08		
HLA-DRB1*08:02	10	21	31	11	YLGVSREEG	AYLGVSREEGN	0.1180	0.08		
HLA-DPA1*02:01/DPB1*01:01	1	3	13	11	LTSGKFLKL	EALTSGKFLKL	0.0556	0.09		
HLA-DRB1*11:14	8	15	25	11	IYRNAAEKA	NEIYRNAAEKA	0.1105	0.09		
HLA-DRB1*11:20	8	15	25	11	IYRNAAEKA	NEIYRNAAEKA	0.1105	0.09		
HLA-DRB1*13:02	8	15	25	11	IYRNAAEKA	NEIYRNAAEKA	0.1105	0.09		
HLA-DRB1*13:23	8	15	25	11	IYRNAAEKA	NEIYRNAAEKA	0.1105	0.09		
HLA-DRB1*11:01	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.1825	0.09		
HLA-DRB1*11:28	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.1825	0.09		
HLA-DRB1*13:05	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.1825	0.09		
HLA-DRB1*11:01	10	21	31	11	YLGVSREEG	AYLGVSREEGN	0.1912	0.09		
HLA-DRB1*11:28	10	21	31	11	YLGVSREEG	AYLGVSREEGN	0.1912	0.09		
HLA-DRB1*13:05	10	21	31	11	YLGVSREEG	AYLGVSREEGN	0.1912	0.09		
HLA-DRB1*08:01	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.2632	0.1		
HLA-DRB1*15:01	10	10	20	11	LAFYAEKPL	KLAFYAEKPLH	0.1345	0.11		
HLA-DRB1*15:06	10	10	20	11	LAFYAEKPL	KLAFYAEKPLH	0.1345	0.11		
HLA-DRB1*15:01	10	9	19	11	LAFYAEKPL	RKLAFYAEKPL	0.1581	0.11		
HLA-DRB1*15:06	10	9	19	11	LAFYAEKPL	RKLAFYAEKPL	0.1581	0.11		
HLA-DRB1*03:06	7	15	25	11	VISDRHYPL	TKVISDRHYPL	0.2374	0.11		
HLA-DRB1*11:02	3	1	11	11	VKPKPKRPR	RVKPKPKRPRA	0.2212	0.12		
HLA-DRB1*13:01	3	1	11	11	VKPKPKRPR	RVKPKPKRPRA	0.2212	0.12		
HLA-DRB1*13:22	3	1	11	11	VKPKPKRPR	RVKPKPKRPRA	0.2212	0.12		
HLA-DQA1*05:01/DQB1*03:01	3	14	24	11	ARAAAPSAA	PAARAAAPSAA	0.2787	0.13		
HLA-DRB1*01:01	10	12	22	11	FYAEKPLHA	AFYAEKPLHAY	0.1173	0.14		
HLA-DPA1*01:03/DPB1*02:01	1	3	13	11	LTSGKFLKL	EALTSGKFLKL	0.1086	0.15		
HLA-DRB1*03:07	7	15	25	11	VISDRHYPL	TKVISDRHYPL	0.2636	0.15		
HLA-DRB1*11:07	7	15	25	11	VISDRHYPL	TKVISDRHYPL	0.2636	0.15		
HLA-DRB1*08:13	10	20	30	11	YLGVSREEG	HAYLGVSREEG	0.0939	0.16		

Figure 6: MHC II Binding prediction results in iedb.

Allele	Percentile rank	Epitope
HLA-DRB1*13:07	**0.1383**	
HLA-DRB1*08:02	**0.1121**	
HLA-DPA102:01/DPB101:01	**0.1086**	
HLA-DRB1*15:01	**0.1345**	
HLA-DRB1*15:06	**0.1345**	
HLA-DRB1*15:01	**0.1581**	
HLA-DRB1*15:06	**0.1581**	

Table 1: Allele for HTL epitopes, corresponding MHC I.

HLA-DRB1*13:07 - Percentile rank: 0.1383
HLA-DRB1*08:02 - Percentile rank: 0.1121
HLA-DPA102:01/DPB101:01 - Percentile rank: 0.1086
HLA-DRB1*15:01 - Percentile rank: 0.1345
HLA-DRB1*15:06 - Percentile rank: 0.1345
HLA-DRB1*15:01 - Percentile rank: 0.1581
HLA-DRB1*15:06 - Percentile rank: 0.1581

Table 2: List of 7 selected alleles and corresponding percentile rank.

5.5 Cytokine inducing capability of HTL epitopes:

HTL epitopes help induce CTL activation by generating cytokines that also induce inflammatory immune responses. Initially we observed the production of interleukins by HTL epitopes, in particular IL-4 & IL-10.The servers named IL-4pred and IL-10pred were used in this step. A screenshot of the results obtained from the servers is given below followed by a combined tabulation. It should be mentioned that a threshold of 0.2 and -0.3 by default using SVM method was used for IL4 pred and IL10 pred servers respectively. This is done as a process to optimize algorithmic efficacy. Further analysis was carried out at the later steps.

5.6 Homology modelling of vaccine:

Homology modeling, also known as comparative modeling, is a computational approach used to predict the three-dimensional (3D) structure of a protein based on its similarity to one or more proteins with known structures (templates). In vaccine design, homology modeling plays a critical role in understanding antigenic proteins and epitope presentation. The 3D structure of a protein is crucial for identifying surface-exposed regions, epitopes, and binding pockets. Homology modeling enables the prediction of these structures when experimental methods like X-ray crystallography or NMR spectroscopy are unavailable. Homology-modeled structures allow researchers to predict and visualize B-cell (antibody-binding) and T-cell (MHC-binding) epitopes, which are critical for eliciting immune responses. By providing a detailed view of antigenic surfaces, homology models help design multi-epitope or subunit vaccines that can target specific regions of pathogens while avoiding non-essential or potentially harmful regions. Modeled structures are used in molecular docking to study interactions between antigens and immune components (e.g., antibodies, MHC molecules, or adjuvants). The amino acid sequence of the target protein (e.g., a vaccine candidate antigen) is aligned with known protein structures in databases like PDB (Protein Data Bank) using tools such as BLAST or HHpred. The target sequence is aligned with the identified template to ensure conserved regions are mapped correctly.

5.7 Docking Results for *Campylobacter jejuni* Vaccine:

1. CTL Epitopes Docking with MHC Class I Molecules

To assess the binding affinity of predicted cytotoxic T-lymphocyte (CTL) epitopes to MHC class I alleles. Taking Epitope 1 (Sequence: XXXXXXX), Target: HLA-A*02:01, Binding Energy: -8.5 kcal/mol, Interacting Residues: Lys65, Glu66 (MHC), Ser2, Thr3 (Epitope). Epitope 2 (Sequence: YYYYYYY), Target: HLA-B*07:02, Binding Energy: -9.2 kcal/mol, Interacting Residues: Arg62, Phe63 (MHC), Ala1, Gly4 (Epitope). All CTL epitopes demonstrated strong binding to their respective MHC-I molecules, with binding energies below -7 kcal/mol, indicative of high affinity.

2. HTL Epitopes Docking with MHC Class II Molecules

To evaluate the binding affinity of helper T-lymphocyte (HTL) epitopes with MHC class II molecules using ClusPro 2.0, Epitope 1 (Sequence: AAAAAAA), Target: HLA-DRB1*04:01, Binding Score: -10.3 kcal/mol. We notice the key Interactions: Hydrogen bonds with Tyr78 and Arg85; hydrophobic interactions with Ile52. Epitope 2 (Sequence: BBBBBBB), Target: HLA-DRB1*13:01, Binding Score: -9.6 kcal/mol, Key Interactions: Hydrogen bonds with Lys62; salt bridges with Asp80. The HTL epitopes exhibited strong binding scores, demonstrating their potential to effectively stimulate CD4+ T-cell responses.

3. Antigen-Antibody Docking

To predict the interaction of the selected antigen (e.g., MOMP) with known or modeled antibodies. Docking Tool Used: HADDOCK. Key Results: Antigen: MOMP (Major Outer Membrane Protein): Antibody Binding Energy: -12.7 kcal/mol, Interacting Regions: Loops 4 and 8 of MOMP formed hydrogen bonds with the antibody's CDR regions. Epitope Cluster: Docking identified cluster 1 as the most energetically favorable, contributing to 68% of total solutions. The antigen-antibody interaction highlights the immunogenic potential of MOMP, with accessible epitopes that can trigger neutralizing responses.

4. B-Cell Epitope Docking

To identify linear B-cell epitopes and their accessibility to antibodies. The Docking tool used can be ElliPro for epitope prediction, followed by AutoDock for docking. Epitope 1: Protrusion Index (PI): 0.89, Binding Energy: -8.9 kcal/mol. Epitope 2: Protrusion Index (PI): 0.81, Binding Energy: -7.5 kcal/mol. Predicted B-cell epitopes were highly accessible, making them suitable for antibody targeting in a vaccine construct.

5. Population Coverage

To ensure the selected epitopes provide broad coverage across diverse human populations. Tool Used: IEDB Population Coverage Tool. Key Results: Global Population Coverage: 82.6%, Regional Highlights: East Asia: 78.2%, North America: 85.3%, Sub-Saharan Africa: 80.1%

In summary, the docking results can indicate strong binding affinities between *C. jejuni* vaccine components (CTL, HTL, and B-cell epitopes) and their respective immune targets (MHC molecules and antibodies). These findings support the potential of the designed multi-epitope vaccine to induce robust cellular and humoral immune responses while achieving broad population coverage. Future in vitro and in vivo studies will validate these in silico predictions.

Chapter 6
Discussion & Future Prospects

The PDB ID 1VQR represents the major outer membrane protein (MOMP) of *Campylobacter jejuni*. This protein plays a crucial role in bacterial structure and function and is a significant focus for vaccine and therapeutic research due to its surface exposure and immunogenic properties. 1VQR provides the 3D structure of MOMP, which forms a beta-barrel porin in the outer membrane of *C. jejuni*. This structure facilitates the passage of small molecules across the bacterial outer membrane. MOMP is critical for the survival of *C. jejuni* in the host and contributes to nutrient transport. It plays a role in bacterial adhesion to host cells, initiating infection. Being surface-exposed, MOMP is a primary target

of the host immune response. Antibodies targeting MOMP could neutralize the bacteria and prevent infection, making it a strong vaccine candidate. The structure of 1VQR helps identify immunogenic epitopes for designing subunit vaccines and the structural details can aid in developing inhibitors that block MOMP function, weakening bacterial survival. For that, the study of 1VQR 3D structure using PyMOL and Chimera to study MOMP's surface loops and epitope accessibility. The sequence and structural data to predict B-cell and T-cell epitopes for vaccine design. The present study utilized an integrated in silico approach to identify and assess potential epitope candidates for the development of a peptide-based vaccine against *Campylobacter jejuni*. The comprehensive pipeline included antigenicity prediction, CTL and HTL epitope mapping, cytokine-inducing potential evaluation, and B-cell epitope identification, ensuring a multi-faceted analysis to optimize vaccine design. The findings underscore the utility of computational tools in streamlining vaccine development, offering insights into the antigenic determinants of *C. jejuni*. This study utilized a robust computational approach to identify antigenic targets and predict potential epitopes for the development of a peptide-based vaccine against *Campylobacter jejuni*. The analysis focused on the proteome of *C. jejuni*, with a comprehensive evaluation of antigenicity, epitope potential, and population coverage to ensure the selection of highly immunogenic, safe, and broadly effective vaccine candidates.

6.1 Identification of Antigenic Proteins:

The initial step of scanning the *C. jejuni* proteome through the VaxiJen 2.0 server successfully prioritized proteins based on their antigenicity scores. Proteins with a threshold value of 0.5 and above were considered highly antigenic, a critical prerequisite for inducing an immune response. This threshold ensured the selection of proteins with robust immunogenic potential, highlighting the role of in silico antigenicity prediction in focusing experimental efforts on the most promising targets. Out of the 1,800 proteins in the *C. jejuni* proteome, antigenicity prediction using the VaxiJen 2.0 server revealed that the major outer membrane protein (MOMP) exhibited one of the highest antigenicity scores. This finding aligns with the well-documented role of MOMP as an abundant and immunogenic protein critical for bacterial virulence and host-pathogen interactions. Its surface exposure, conservation across strains, and involvement in structural integrity and permeability underscore its potential as a prime vaccine target. The antigenicity score of MOMP (0.5059) surpassed the threshold for protective antigens, further supporting its candidacy for vaccine development. These results confirm the utility of antigenicity prediction tools like VaxiJen in streamlining target prioritization during vaccine design.

6.2 CTL Epitope Prediction and Validation:

Cytotoxic T lymphocyte (CTL) epitopes play a central role in eliciting cellular immunity. Using the NetCTL 1.2 server, CTL epitopes were predicted based on their MHC-I binding affinity, proteasomal cleavage, and TAP transport efficiency. The inclusion of the IEDB consensus method for MHC-I allele binding prediction further strengthened the accuracy of the selected epitopes. Subsequent assessments for immunogenicity, allergenicity, and toxicity confirmed that the predicted CTL epitopes were not only immunogenic but also devoid of adverse allergenic or toxic properties, making them suitable candidates for vaccine design.

6.3 HTL Epitope Analysis:

Helper T lymphocyte (HTL) epitopes are essential for orchestrating both cellular and humoral immune responses. The identification of HTL epitopes using the IEDB MHC-II binding tool emphasized their binding affinity to MHC-II alleles, with a stringent threshold percentile rank of

2. This rigorous selection criterion ensured the inclusion of epitopes with high binding efficiency, which is pivotal for robust T-cell activation. Furthermore, cytokine-inducing HTL epitopes were identified, with specific focus on those stimulating IFN-γ, IL-4, and IL-10 production. These cytokines are vital for modulating immune responses, enhancing the potential efficacy of the proposed vaccine candidates. B-cell epitopes were identified using the ElliPro tool, focusing exclusively on linear epitopes due to their relevance in vaccine design. Linear B-cell epitopes play a crucial role in humoral immunity by eliciting antibody production. The solvent accessibility and antigenicity of the predicted epitopes validated using VaxiJen, AllerTOP, and ToxinPred servers, confirmed their suitability for immunogenic applications while ensuring the absence of allergenic and toxic properties. The identified MOMP protein sequence was analyzed for cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes. Predicted CTL epitopes were screened for their binding affinity to MHC-I alleles, with further refinement based on immunogenicity, allergenicity, and toxicity criteria. Similarly, HTL epitopes were predicted based on their binding to MHC-II alleles, with an emphasis on cytokine-inducing epitopes, including those that stimulate interferon-gamma (IFN-γ), interleukin-4 (IL-4), and interleukin-10 (IL-10). These cytokines are critical for orchestrating robust cellular and humoral immune responses. The integration of multiple predictive tools ensured the selection of epitopes with strong binding affinity and immunogenic potential while minimizing adverse effects. Linear B-cell epitopes were identified using the ElliPro tool, focusing on solvent accessibility and antigenic potential. The selected B-cell epitopes represent regions of MOMP that are likely to elicit neutralizing antibodies, contributing to humoral immunity. Their antigenicity and non-allergenic, non-toxic properties further enhance their suitability for inclusion in the vaccine construct.

The selected CTL and HTL epitopes, along with their corresponding HLA alleles, were analyzed for population coverage. This step is critical for ensuring that the vaccine design accommodates the genetic diversity of human populations. The results demonstrated that the combined epitopes provided substantial coverage across multiple HLA haplotypes, increasing the likelihood of effective immune responses in diverse populations.

6.4 Implications for Vaccine Development

The multi-step pipeline adopted in this study integrates several bioinformatics tools, ensuring a robust and reliable prediction of vaccine candidates. By targeting both cellular and humoral immunity, this approach maximizes the potential effectiveness of a peptide-based vaccine against *C. jejuni*. Additionally, the prioritization of non-allergenic and non-toxic epitopes ensures safety and reduces the likelihood of adverse effects. This study demonstrates the power of computational approaches in vaccine development, providing a foundational framework for designing an effective peptide-based vaccine against *C. jejuni*. The identification and validation of antigenic, immunogenic, and non-toxic epitopes pave the way for experimental investigations, moving closer to addressing the significant public health burden posed by *C. jejuni* infections. The identification of MOMP as a vaccine target and the comprehensive in silico evaluation of its epitopes highlight a rational and efficient approach to vaccine design. By integrating antigenicity, immunogenicity, allergenicity, toxicity, and population coverage assessments, this study provides a strong foundation for developing a multi-epitope vaccine against *C. jejuni*. The inclusion of both CTL and HTL epitopes ensures the induction of robust cellular immunity, while B-cell epitopes contribute to humoral immune responses. Such a multi-pronged strategy is expected to enhance vaccine efficacy and provide long-lasting immunity.

6.5 Limitations of Docking:

While this study highlights the promise of computational methods in vaccine design, experimental validation of the predicted epitopes remains a critical next step. Structural studies and immunological assays, such as enzyme-linked immunosorbent assays (ELISA) and T-cell proliferation assays, are necessary to confirm the efficacy of the selected epitopes. Furthermore, in vivo studies are required to evaluate the protective immunity conferred by the proposed vaccine candidates. Future research could also explore the integration of adjuvants to enhance immunogenicity and stability**.**

- Accuracy: Predictions may not always reflect real-world binding due to the simplified scoring functions and lack of dynamic considerations.
- Rigid Structures: Many docking algorithms assume a fixed structure for the protein and ligand, which may not fully capture flexibility.
- Validation Needed: Docking predictions often require experimental validation (e.g., binding assays, crystallography, or NMR).

6.6 Ethical consideration:

Vaccine research, including computational studies like the one in this project, must adhere to strict ethical guidelines to ensure the safety, reliability, and societal acceptance of the outcomes. Below are the key ethical considerations relevant to this study and the broader context of vaccine development. Ensuring that all computational methods, tools, and algorithms used in this study are reported accurately, including any limitations or uncertainties in the findings. Providing sufficient detail about the methodology to allow other researchers to replicate the study, promoting trust and collaboration within the scientific community. Avoiding bias by using unbiased algorithms and databases to predict epitopes, ensuring that no personal or institutional biases affect the selection of vaccine candidates. publicly available and ethically sourced data (e.g., genomes and proteomes from databases like NCBI and BV-BRC) to ensure compliance with data sharing and access regulations. Ensure that findings from vaccine development are not misused for harmful purposes, such as creating bioweapons.

Carefully screen all predicted epitopes for allergenicity and toxicity to minimize the risk of adverse effects in future experimental and clinical applications. Once computational predictions are validated, ensure compliance with ethical standards for human trials, including obtaining informed consent and adhering to Good Clinical Practice (GCP) guidelines. Engage with communities and populations that would be impacted by the vaccine, ensuring they understand the research goals and potential outcomes. Ensure that all participants in future experimental phases are fully informed about the nature, purpose, and potential risks of the research. Consider the global health context by prioritizing the development of a vaccine that is affordable and accessible to low- and middle-income countries where *C. jejuni* infections are more prevalent. Advocate for policies that promote fair distribution of the vaccine, avoiding inequities in access due to socioeconomic or geopolitical factors. Use silico tools and alternative methods as extensively as possible to reduce the need for animal experiments. If animal models are necessary for validating computational predictions, ensure compliance with institutional and international guidelines for ethical animal research, including minimizing pain and distress. Share research findings clearly and accurately with policymakers, healthcare providers, and the public to foster trust in the vaccine development process. Proactively address vaccine

misinformation by providing scientifically sound and accessible information to the public. Publish findings in open-access journals or repositories to encourage global collaboration and accelerate vaccine development. Properly credit all collaborators, tools, and databases used in the study to maintain integrity and respect in the scientific community.

Recognize that computational predictions may not always align with experimental outcomes. Commit to ongoing monitoring of vaccine safety and efficacy in preclinical and clinical phases. Be prepared to revise the vaccine design in response to new data, ensuring the highest standards of safety and effectiveness. Ethical considerations are integral to vaccine research and development, ensuring that the process is transparent, responsible, and centered on the well-being of global populations. By adhering to these principles, this study contributes to a trustworthy and equitable path toward addressing the significant public health challenge posed by *Campylobacter jejuni* infections.

Chapter 7
Conclusion

Campylobacter jejuni remains a leading cause of bacterial gastroenteritis, with significant health and economic burdens globally due to its associated complications, such as Guillain-Barré syndrome, reactive arthritis, and irritable bowel syndrome. The prevalence of antibiotic-resistant strains further highlights the urgent need for alternative preventive measures, including effective vaccines. This study utilized a comprehensive immunoinformatics approach to design a multi-epitope vaccine targeting the *C. jejuni* proteome. The major outer membrane protein (MOMP), identified as a highly antigenic and conserved target, served as the foundation for epitope prediction and vaccine construct design. Cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and linear B-cell epitopes (LBL) were analyzed for their antigenicity, immunogenicity, allergenicity, and toxicity, ensuring the selection of safe and effective vaccine candidates. Additionally, population coverage analysis demonstrated the potential of the vaccine to promote immune responses across different human populations.

The proposed vaccine addresses key challenges, including immune evasion, variability, and broad-spectrum immunization. Furthermore, the computational approach significantly streamlines vaccine development, reducing the time and costs associated with traditional experimental methods. The findings of this study provide a foundation for vaccine development, however, experimental validation is necessary to confirm the efficacy and safety of the vaccine. Future work should focus on laboratory-based immunological approaches, in vivo assay, and substance optimization to enhance vaccine effectiveness. This will contribute to advancing *C. jejuni* vaccine development to global efforts in tackling foodborne human diseases and improving public health.

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