



Identification of B cell and T cell specific peptide vaccine for *Moraxella catarrhalis*.

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Abstract :

Introduction:

Moraxella catarrhalis is a gram-negative bacterium that commonly causes respiratory tract infections, particularly in individuals with compromised immune systems or underlying respiratory conditions. In recent years, there has been growing interest in developing peptide-based vaccines to target specific antigens of *M. catarrhalis* for B cell and T cell immune responses.

Materials and methods:

Moraxella catarrhalis, a heat shock protein (hsp65) gene, complete cds. Amino acid sequence for the above entry was retrieved from the NCBI database. Fasta sequences were used for B cell and T cell epitope prediction. Bepipred Linear epitope Prediction 2.0 online server program was used to predict the B cells immunogenic epitopes MHC-1 binding promiscuous T cell epitopes were identified using IEDB analysis resource with HLA allele reference set. The resulting epitopes

CC License CC-BY-NC-SA 4.0	were analyzed. Results: Peptide LGEQKESPFDALSER was selected as it was homologous (100%)to the Moraxella Catarrhalis and the length was 15 mers appropriate as a candidate vaccine. .MHC 1 bindingT cell epitope prediction was carried out by using IEDB analysis resource online server program. Discussion: Peptide LGEQKESPFDALSER was selected as it was homologous (100%)to the Moraxella Catarrhalis and the length was 15 mers appropriate as a candidate vaccine.MHC 1 bindingT cell epitope prediction was carried out by using IEDB analysis resource online server program. Conclusion: The identification of B cell and T cell specific peptide vaccines for Moraxella catarrhalis is a multi-step process aimed at understanding the immune response against the pathogen and designing a targeted vaccine. By characterizing the immune response, researchers can identify the specific antigens and epitopes that trigger an immune response in infected individuals. Keywords: Moraxella catarrhalis,peptide vaccine,B cell epitopes,T cell epitopes,Universal heath, Diseases, Well being, Health, International Health policy.
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Introduction:

Moraxella catarrhalis is a gram-negative bacterium that commonly causes respiratory tract infections, particularly in individuals with compromised immune systems or underlying respiratory conditions(1) .In recent years, there has been growing interest in developing peptide-based vaccines to target specific antigens of M. catarrhalis for Bcell and T cell immune responses.B cells and T cells play crucial roles in the adaptive immune response. B cells produce antibodies that can recognize and bind to specific antigens, while T cells recognize and destroy cells infected with pathogens or provide help to other immune cells(2).By identifying B cell and T cell specific peptides derived from M. catarrhalis antigens, it is possible to design peptide vaccines that can induce a targeted immune response against the bacterium.

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In addition, up to 2.5–10% of adults with stable COPD have lower respiratory tract colonizations by *M. catarrhalis*. Due to highly inflammatory bacterial cell wall antigens sloughing into the airway, lower airway colonization increases airway inflammation in COPD patients(3). A vaccine that targets exacerbations in adults with COPD as well as otitis media in children is necessary due to the major clinical implications of *M. catarrhalis*. Since it is a non encapsulated bacterium, *M. catarrhalis* doesn't release exotoxins. Many outer membrane proteins (OMPs) have been the main focus of recent vaccine studies as potential vaccines(4). Thus far, only a small number of OMPs have been investigated and are presently undergoing various stages of assessment as part of an endeavor to create a multicomponent vaccine against *M. catarrhalis*.

Although *M. catarrhalis* is generally thought to be a commensal limited to humans, research conducted in the last few years has shown that it is now a significant infectious agent in the upper and lower respiratory tract and the cause of roughly 17% of pediatric acute otitis media infections(5). Moreover, *M. catarrhalis* has been linked to various infections, including meningitis, endocarditis, and bacteremia, particularly in patients with compromised immune systems(6). The creation of a potent vaccine is a top health priority because *M. catarrhalis* has shown signs of rising antibiotic resistance in recent years. Here, we present a newly developed multipeptide vaccine that is based on the mapped epitopes of vaccine candidates that have been culled from the entire *M. catarrhalis* proteome(7).

While there isn't currently an FDA-approved *M. catarrhalis* vaccine, there are several stages of development for them. *M. catarrhalis* adhesive proteins have been extensively studied as possible vaccine candidates (8). These are proteins found on the outside of the membrane that enable the microbe to attach to its target receptor in the respiratory system of humans, marking the beginning of bacterial pathogenesis (9). When administered to animal models, this vaccine improved the removal of bacteria from the lungs(10).

Materials and methods :

Moraxella catarrhalis, a heat shock protein (hsp65) gene, complete cds. Amino acid sequence for the above entry was retrieved from the NCBI database. Fasta sequences were used for B cell and T cell epitope prediction. Bepipred Linear epitope Prediction 2.0 online server program was used to predict the B cells immunogenic epitopes MHC-I binding promiscuous T cell epitopes were identified using IEDB analysis resource with HLA allele reference set. The resulting epitopes were analyzed.

Prior to epitope prediction, the SignalP-5.0 Server was used to predict the presence and location of signal peptides based on the amino acid sequence of filtered proteins. The mature polypeptides that were thus obtained were then examined using the Immune Epitope Database's prediction tools (IEDB). While IEDB recommended a 2.22 prediction method and a full HLA reference set that covers >99% in terms of population coverage for analysis of MHC-II binding, MHC-I binding was detected by applying NetMHCpan EL 4.0 as a prediction method and an

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HLA allele reference set that provides >97% in terms of population coverage. Using IEBD, antibody epitope prediction was also carried out.

M. catarrhalis BBH18's entire proteome was obtained This strain was chosen because it is the NCBI reference M. catarrhalis strain. The entire proteome of M. catarrhalis was subjected to protein prioritization using the reverse vaccinology technique. First, the Geptop 2.0 web server was used to identify essential proteins, which are necessary for M. catarrhalis to survive and have an essentiality cutoff of 0.24. By comparing the orthology and phylogeny of query proteins with the datasets defined experimentally in the database of essential genes (DEG) ,this server finds essential proteins. Subsequently, the subcellular localization of the essential proteins was examined using the online server . The identification of the exoproteome was the ultimate result of this filtration process. These proteins were used to calculate the potential for virulence. After being filtered out in the previous step, the proteins were searched against the human proteome using BLASTp from the NCBI. Proteins with $\geq 35\%$ identity were removed because they were thought to identified transmembrane helices in the proteins from the previous step, predicted the molecular weight of the proteins.

Results:

Peptide LGEQKESFPDALSER was selected as it was homologous (100%)to the Moraxella catarrhalis and exhibits antigenic diversity, making the development of traditional vaccines challenging. Peptide vaccines offer a targeted strategy by focusing on specific epitopes that are conserved and immunogenic. This approach is particularly relevant for a bacterium with variable surface antigens.

The table 1 shows the peptides identified by the program considering many factors including amino acid composition, their length,sequence number, score, percentile mark, etc. Table 2 shows B cell response. Peptide (9mer or 10mer) induces a good T cell immune response. Among these, peptides around 15-25 mer induce ideal B cell response.

Table 1:Predicted B cell peptide

No	Start	End	Peptide	Length
1	71	74	KQFN	4
2	88	89	QP	2

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3	104	104	T	1
4	106	110	GTPLD	5
5	121	121	A	1
6	124	129	RYFSHD	6
7	132	132	E	1
8	139	153	LGEQKESPFDA LSE R	15
9	166	169	STQQ	4
10	176	183	VSKTVNT	8
11	186	195	YRIFDKLGID	10
12	209	209	L	1

Figure 1: B cell response recording immunogenicity and non immunogenic response

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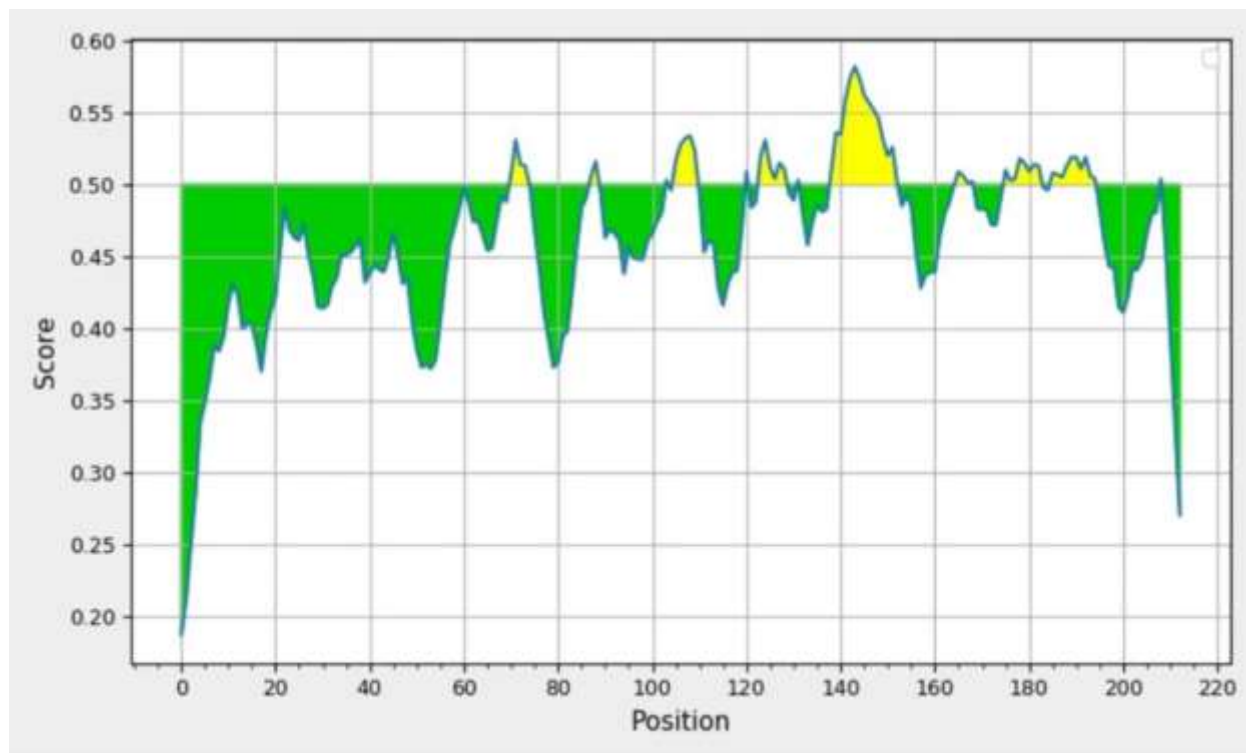


TABLE 2: T cell response to identify ideal T cell peptide

allele	seq_num	start	end	length	peptide	score	percentile_rank	
HLA-B*40:01	3	29	37	9	AEQLAE TVL	0.982634	0.01	
HLA-A*68:01	3	42	51	10	ESPFDAL SER	0.967207	0.02	
HLA-A*02:06	2	27	35	9	KQFNPN KV	0.942316	0.02	
HLA-A*31:01	4	19	27	9	KTVNTY RYR	0.941137	0.01	

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HLA-A*68:01	2	16	24	9	NINGVE ATR	0.909744	0.07	
HLA-A*03:01	2	43	52	10	AQPYP SMLLK	0.908978	0.03	
HLA-B*08:01	2	30	38	9	NPNIKV LAV	0.889342	0.02	
HLA-B*58:01	4	16	24	9	VSAKTV NTY	0.887623	0.07	
HLA-B*57:01	4	16	24	9	VSAKTV NTY	0.884592	0.13	
HLA-A*68:01	4	17	25	9	SAKTVN TYR	0.882423	0.1	
HLA-B*15:01	2	50	58	9	LLKAGV NGY	0.881509	0.03	
HLA-A*31:01	4	17	25	9	SAKTVN TYR	0.873936	0.04	
HLA-A*02:03	3	47	55	9	ALSERE KQV	0.853015	0.04	
HLA-A*11:01	2	43	52	10	AQPYP SMLLK	0.837635	0.06	
HLA-A*30:02	4	16	24	9	VSAKTV NTY	0.830678	0.02	
HLA-B*40:01	3	49	57	9	SEREKQ VAM	0.827877	0.1	

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HLA-B*15:01	4	16	24	9	VSAKTV NTY	0.825778	0.05	
HLA-A*02:01	1	21	30	10	MLDDDP NIEV	0.800869	0.08	
HLA-A*02:01	1	20	28	9	RMLDDD PNI	0.792584	0.08	

In the B cell response graph, the part highlighted in yellow shows immunogenic response and the part highlighted in green shows non-immunogenic response. Then we have represented the peptides for immunogenic response in the form of a table. The ideal peptide should be of length 20 mer to induce B cell response. From the peptides obtained we can say that, the peptide LEQKESFPDALSER was selected as it was homologous (100%) to the *Moraxella catarrhalis* and exhibits antigenic diversity, making the development of traditional vaccines challenging. Peptide vaccines offer a targeted strategy by focusing on specific epitopes that are conserved and immunogenic. This approach is particularly relevant for a bacterium with variable surface antigens.

Discussion:

Traditional methods of developing vaccines, like attenuated or killed vaccines, take a long time because it can take decades to create a vaccine that works(11). Furthermore, the fact that many microorganisms are hard to grow or weaken and cause unfavorable immune reactions supports the need for a radical change in the methodology used to develop vaccines(12). In recent times, there has been a shift in the vaccine development process towards a more efficient and time-efficient genome pre-screening approach. The antigenic diversity of *Moraxella catarrhalis* makes the creation of conventional vaccines difficult(13). By concentrating on particular, conserved, and immunogenic epitopes, peptide vaccines provide a targeted approach. This strategy is especially pertinent for bacteria that have changing surface antigens(14).

The pathogenic bacteria *Moraxella catarrhalis* is linked to otitis media, respiratory infections, and flare-ups of chronic obstructive pulmonary disease (COPD). Creating a peptide vaccine may be a useful tactic in the fight against *M. catarrhalis* infections. The search for peptides specific to B cells centers on epitopes that stimulate the production of antibodies. These antibodies have the ability to increase phagocytosis, prevent adhesion, and neutralize bacterial toxins. Peptides unique to T cells stimulate cellular immunity. While CD8⁺ T cells have the ability to directly destroy infected cells, CD4⁺ T cells support B cells and other immune cells. Both arms of the

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immune system should be stimulated by a comprehensive vaccine. It is essential to choose important antigenic proteins for *M. catarrhalis*(3). Epitope mapping is a technique that uses experimental assays and bioinformatics tools to find regions that interact with B cell receptors (BCRs) or are presented in major histocompatibility complexes to T cells.

The pathogenic bacteria *Moraxella catarrhalis* is linked to otitis media, respiratory infections, and flare-ups of chronic obstructive pulmonary disease (COPD). Creating a peptide vaccine may be a useful tactic in the fight against *M. catarrhalis* infections. The search for peptides specific to B cells centers on epitopes that stimulate the production of antibodies. These antibodies have the ability to increase phagocytosis, prevent adhesion, and neutralize bacterial toxins. Methods such as peptide microarrays, phage display, or epitope prediction algorithms are used to identify linear and conformational B cell epitopes within particular proteins. B cell epitopes with high affinity are essential for strong antibody responses. Potential T cell epitopes can be identified with the help of peptides' binding to MHC molecules. This includes MHC binding affinity prediction algorithms or MHC tetramer staining assays. Functional assays are necessary for T cell epitope validation.

B cell and T cell epitopes are combined into a single construct in the development of a peptide vaccine. This guarantees a strong and well-coordinated immune response. For long-term protection, it is essential to ensure the induction of immunological memory. After a subsequent exposure, memory B cells and memory T cells can quickly and effectively respond by remembering details about the pathogen. Antigenic variation in *M. catarrhalis* could pose a challenge to the long-term stability of vaccine efficacy. Differences in the host immune response between individuals can affect how effective the vaccine is. Animal models are used in preclinical research to evaluate the safety, immunogenicity, and protective effectiveness of vaccines. Clinical Examinations: In order to proceed to clinical trials, the vaccine must first be tested in humans for efficacy, safety, and immunogenicity (15). Various stages evaluate increasing

To trigger a humoral immune response, B cell-specific peptide antigens must be identified. These peptides ought to have the ability to stimulate the synthesis of antibodies that are opsonized or neutralized against *Moraxella catarrhalis*, thereby blocking the pathogen's colonization and spread(16). Cellular immunity requires the activation of T cell responses. Strong cellular reactions, such as the activation of cytotoxic T cells and the development of immunological memory, can be elicited by T cell-specific peptides. This improves the host's capacity to identify and eradicate *Moraxella catarrhalis* in the event of another exposure(17). The genomic and proteomic analysis of *Moraxella catarrhalis* strains serves as the foundation for identifying potential antigenic targets. Examining the bacterial surface proteins and secreted proteins aids in pinpointing candidates that interact with the host immune system. Utilizing bioinformatics tools to predict B cell and T cell epitopes is a critical step(18). The prediction

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algorithms help identify regions of proteins that are likely to induce strong immune responses. This step ensures a rational and targeted approach to peptides (19).

Designing synthetic peptides based on predicted B and T cell epitopes is a practical application of the study's findings. These synthetic peptides can be manufactured for further in vitro and in vivo evaluations, serving as potential components of a peptide vaccine. The identification of B and T cell-specific peptides is expected to contribute to the development of a highly effective Moraxella catarrhalis vaccine (20). Targeting both arms of the immune system increases the likelihood of a robust and comprehensive immune response, providing protection against vaccines. Focusing on conserved and immunogenic epitopes has the potential to provide broad protection against diverse Moraxella catarrhalis strains (21). This is a significant advantage, especially considering the variability in surface antigens observed in this bacterium. The study's findings may contribute to broader knowledge on vaccine design strategies, especially for pathogens with antigenic variability. Lessons learned from this research could potentially be applied to the development of peptide vaccines against other infectious agents (22).

The identified peptides must undergo rigorous validation and testing, including in vitro assays and animal models, to confirm their immunogenicity and protective efficacy. Transitioning from preclinical studies to clinical trials is a critical step in assessing the safety and effectiveness of the peptide vaccine. The potential variability in Moraxella catarrhalis strains should be considered. Continued surveillance and adaptation of the vaccine to cover prevalent strains will be essential.

Conclusion:

The identification of B cell and T cell specific peptide vaccines for Moraxella catarrhalis is a multi-step process aimed at understanding the immune response against the pathogen and designing a targeted vaccine. By characterizing the immune response, researchers can identify the specific antigens and epitopes that trigger an immune response in infected individuals. This knowledge is then used to map B cell and T cell epitopes, which are essential for designing vaccines that stimulate the production of antibodies and activate cellular immune responses. The ultimate goal is to develop a peptide vaccine that incorporates the identified B and T cell epitopes, which can elicit a robust immune response against Moraxella catarrhalis.

Conflict of interest:

The author declares that there were no conflicts of interests in the present study.

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