

## Identification of Vaccine targets in *Klebsiella pneumonia* using Violin

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### Abstract:

*Klebsiella pneumoniae* causes community acquired, nosocomial infections & other diseases like bacteremia, hepatic abscess, meningitis, respiratory & urinary tract infections worldwide. This organism is rod shaped, gram-negative, non-motile encapsulated, lactose fermenting facultative anaerobic bacterium, found as the normal flora on the mouth, skin and intestine. *Klebsiella pneumoniae* belonging to the Enterobacteraceae family typically expresses 2 types of antigens -O antigen a component of Lipopolysaccharide & -K antigen a capsular polysaccharide. Many antibiotics are recommended for the treatment, but the organism is showing resistance making the treatment challenging. The solution could be use of vaccines; a polyvalent pneumococcal polysaccharide vaccine was licensed for the treatment of pneumococcal infections during late 1977, but there were reports of its failure. VIOLIN a web-based vaccine tool integrates vaccine literature data mining, vaccine research data curation & storage & curated vaccine data analysis for vaccines & vaccine candidates developed against various pathogens of high priority in public health & biological safety. VIOLIN also provides a program for vaccine target prediction. In our study we have used this tool for the detection of vaccine targets for *K.pneumoniae* NTUH-K2044 and it was found that the putative outer membrane can be synthesized in vivo and can be used as a potent protein sub-unit protein against *Klebsiella* infections.

**Keywords:** Antibiotics, vaccine, polysaccharide, VIOLIN

### Introduction:

The start of the “vaccine” by Jenner’s description of small pox has revolutionized the world of immunology in preventing infectious diseases (E. Jenner 1798) [1]. Critical advances in the field of vaccine took place in 19<sup>th</sup> and early 20<sup>th</sup> century by the scientists like **Pasteur, Koch, Von Behring, Calmette, Guerin and Ehrlich** by the development of antiserum, antitoxins and live- attenuated vaccines (M. R. Hilleman 2000) [2]. Further advances in cell cultures, molecular biology and immunology have

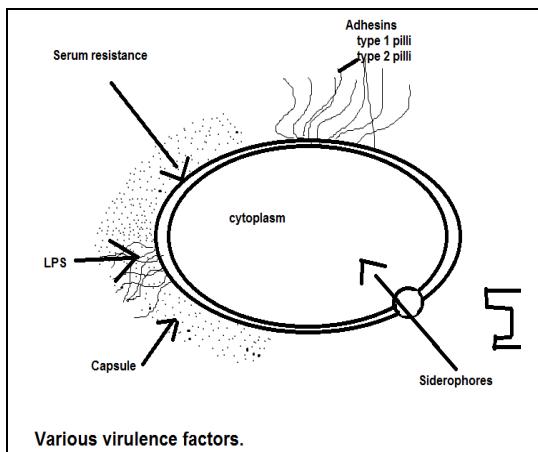
led to the modern-era of “subunit” vaccine development. The **recombinant hepatitis B vaccine** is one of the first licensed sub-unit vaccines for human use. The advent of computers and informatics has facilitated vaccine-research and development. **ImmunoInformatics** targets the mathematical and computational approaches to address immunological questions. Since 1980’s many ImmunoInformatics methods have been used to predict **T-Cells** and **B-Cells immune epitopes**, [3]. The experimentally verified immune epitopes are stored in web-based databases like the **Immune Epitope Database and Analysis Resource** (IEDA) and few others [4].

The molecular and sequencing technologies, bioinformatics analysis has allowed *in silico* selection of vaccine targets [5]. A novel approach called **REVERSE VACCINOLOGY** or **IMMUNOME DERIVED VACCINE DESIGN** [6]. This algorithm was first used against *Neisseria meningitidis* for the identification of vaccine targets in it [7]. The **Vaccine Investigation OnLine Information Network** (VIOLIN) the first ImmunoInformatics tool for vaccine design was developed in 1980’s by **DeLisi and Berzofsky** and others [8], it is a web based central resource, allowing easy curation, comparison and analysis of vaccine-related research data. VIOLIN is publicly available at <http://www.violinet.org> contains information regarding microbial pathogenesis, host ranges, host specificities, as well as vaccine – specific information such as vaccine type, methodology, genetic engineered genes.

**Klebsiella pneumonia** is one of the species of the genera KLEBSIELLA, and is a gram negative facultative anaerobe belonging to the phylum **PROTEOBACTERIA**, and exhibits close genetic resemblance to the other bacteria of Enterobacteriaceae like Escherichia, salmonella, shigella, and Yesinia [9]. The community acquired Pyogenic Liver Abscesses (PLA) is caused by *Klebsiella pneumoniae* complicated with metastatic meningitis and endophthalmitis have been found globally [10]. For the study of vaccine targets in the genus Klebsiella we have chosen the strain Klebsiella

*pneumoniae* NTUH-K2044. The sequencing for this organism was done by **Keh-Ming Wu, Ling-Hui Li, et al** at the **Institute of Biomedical Informatics, Taiwan**. This organism was found to have the circular chromosome and the length of genome is **52, 48,520 kb** with an extra-chromosomal circular plasmid of length **2, 24,152 kb** by name PK2044. The complete genome of this strain is available in the NCBI genome under the accession number NC\_012731.

The bacterium overcomes the innate immunity through several means; the **capsule** which is the first pathogenic determinant is also called **K antigen**. The capsule is composed of complex acidic polysaccharides which is so massive that it prevents the phagocytosis by **PolyMorphoNuclear (PMN)** granulocytes and also prevents the cell death by the bactericidal serum mediated complement proteins of the host<sup>[11]</sup>.



Based on the symptoms and tests the physician may confirm that the patient is suffering from the Klebsiella infections and may suggest the drugs like Cefotaxime Carbapenem Ceftizoxime Gentamicin Tobramycin which have bactericidal activity of inhibiting the cell wall synthesis by binding to the **Penicillin Binding Proteins (PBP)** which inhibits the peptidoglycan synthesis.

The prudent use of antibiotics has led to the resistance of the antibiotics by the microbes as said by Dr.Dilip Mathai, the President of Clinical Infectious Diseases Society. This is an unusual nature of Klebsiella to show resistance to structurally unrelated antibiotics like nalidixic acid, timethoprim

and chloramphenicol and is also known to have developed resistance to the class of antibiotics known as **Carbapene** and has developed into a multidrug-resistant mutant.

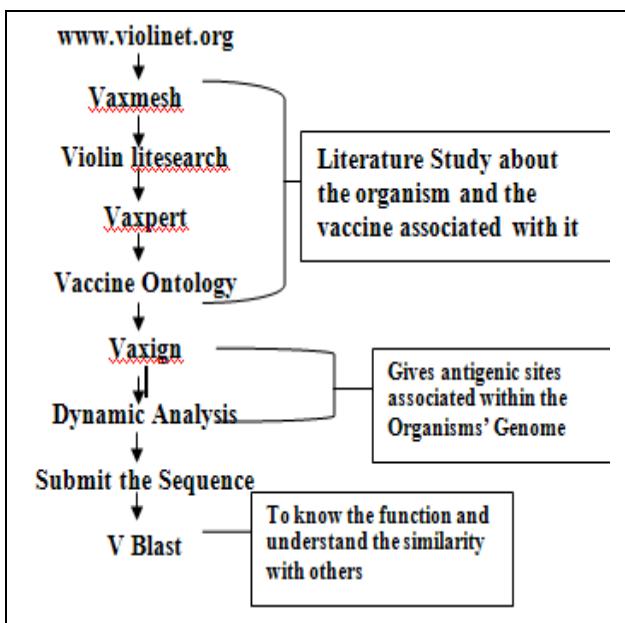
The Klebsiella species bearing resistance to this class of antibiotic are called **KPC producing Organisms or Carbapenem resistance Klebsiella pneumoniae (CRKP)**. These organisms produce an enzyme **carbapenemase** which helps in drawing resistance against Carbapenem and the study on the multiresistant CRKP was done in Los Angeles Country by the Department of Public Healthcare.

#### Materials & Methodology:

Klebsiella pneumoniae NTUH-K2044's genome. Violin tool- the database cum search tool for vaccine. From the literature study it has been found that there are many factors which could trigger immune response in humans. Some of the antigenic determinants that could be used as the vaccine targets are the cell membrane components, fimbriae, pili, virulence factors and drug resistant factors and others. As the identification of vaccine targets include the identification of antigenic sites that get exposed or triggers the immune cells to present these antigens to the MHC molecules for further processing and elimination of the antigen.

We have obtained the genome of the organism, which was later translated by the translation tools available online at the emboss site and it can be easily accessed by <http://www.ebi.ac.uk/Tools/emboss/transeq/>. So we have submitted the protein sequences from the Klebsiella pneumoniae NTUH-K2044 genome to the Vaxign system in the fasta format for the prediction of its adhesion probability, p-values of the sequences that bind to the class I and II epitopes in humans. The procedure used is depicted in the flow chart below:

The submission of the protein sequences to the VIOLIN's Vaxign provides with the result page which gives us the details like the adhesion probability, the protein's localization and the type of MHC molecule that can bind to the given virulent sequence.



The submission of the protein sequences to the VIOLIN's Vaxign provides with the result page

#### Vaxign: Vaccine Target Prediction and Design

##### Sequence Alignment

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>614876HHGXLRYSGKHRGEIPRRTRHGRRENRSVQPAVPVQYRDXSAEPAACADRLQTRVRPEPAG
EKHAAVAVPXRDXILXKHAYSGPRAEHSQTDXASQPAGSDVPRVXSAIDGDDPVCRVPERAARSDGRSV
SRDGPRQRQHDPAVSSGSAAGDAGDXDDHRREPSGAGVPADA VRNRRPLSRLRNRYPRLSSGGWSLH
RXSAERREXQVIDAHQGSGLPLPFFTLQGEHMKTSPQRAIYYIVAIQIWEYFSFYGMRALLILYLTHQLGF
DDSHAIISLFSAYASLVYVTPILGGWLADRLGNRTAVIAGALLMTLGHVVVLGVESTSAWSLYVALAIIICGY
GLFKSNISCLLGELYAHDDP RRDGGFSLLYAAGNVGSIAAPIACGLAAQWYGWHIGFALAGIGMFIGLMIFL
SGSRHFRHTRGVDKPALRAVKFvlptwgwlLVMCLAPVFFTLLQNNWSGYLLAIVCLFAAQMVARI MIKAP
EHRRALWQIVLLMLAGTLFWVLAQQGGSSISLF
  
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The highlighted sequence is a predicted epitope that binds to human MHC class I (HLA-A2.1) with a p value 0.0337. On submission of the entire genome to the Vaxign, it was found that a sequence with a p value of 0.0337 would bind to MHC class I in humans. Upon verification of the sequence it was found that **it is the protein sequence that codes for the outer membrane** and the verification was done by the use of the local alignment tool the BLAST. This BLAST is the Basic Local Alignment Search Tool available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

which gives us the details like the adhesin probability, the protein's localization and the type of MHC molecule that can bind to the given virulent sequence.

##### Results and Discussions:

The highlighted sequence below is a predicted epitope that binds to human MHC class I (HLA-A2.1) with a p value 0.0337. On submission of the entire genome to the Vaxign, it was found that a sequence with a *p value* of 0.0337 would bind to MHC class I in humans. Upon verification of the sequence it was found that it is the protein sequence that codes for the outer membrane and the verification was done by the use of the local alignment tool the BLAST. This BLAST is the Basic Local Alignment Search Tool available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. This alignment tool works on principle of heuristic algorithm to identify the locally aligned sequences between the given query and the sequences present in the database.

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**Vaxign: Vaccine Target Prediction and Design**

**Sequence Alignment**

>640543QSALYXWYYLEKLRXRVFQSICQHCCIQWPWPGHSYRQELXNSTTDCSVYLVCFLHCILASVPVLY  
GLFPGAGRASVYLDGFLRVKTVHSRSPCLRWVLAGHDGEKPLSPRQSMQGRATPACMADVXFTRVCLTI  
TVARSGIRYGRYYEYXLCPLQRRATTQTRXAAQFTPSQRYRLILPCCHAPGAPXCXGVESSTGNQCDSFGTR  
RRHPGGYLPAAAGXXSPCQGXALFNQNHPKISRIRCGQKQSXSGDVAGGXNQPASRQICXGCKSYHSLPG  
LPVRNSASGLPAGDFTGPXPYDPRYXANSLPGVHLANPCHSXIYPAGNGYRPLAQGDNXAPAGYQSDKT  
GCXTAETGTNRRNHDAVVAQXXSESXRKFKEKISAHPGKXLSCXRQYPVHQXSRSRXCSWAQTQLSR  
AFTINFVQRTGTVXSVKKLRKRXKAECARRCXXKISFCPAPYRAGGSQGVKMSRLTGICEADPARRLYKV  
AVSRGNTFLSAKERFRVQVCFKNAITHPDEDRLAELLTVHHFLADTSILWHMEQKYACTTTWATTSTW  
LPEIYQRQASTPNVVKIIKFMHARFPGLKTVVAETNEQGYEQALTSDVFEIPYWPVLPKFESDIGIXVTIAGM  
IRYTETMSNKDKCKTLDLMIKSFKSGLIPIEKMLSKTERLIPLNINTAGKIFTHATWPRCRYIFVPDEAGIMK  
LVNVVKLRLKPSEEKXLTRFSCXXLLFVHSDIIIXQTKSVPVRVXLTRVEALMYTPYGVSHRSSFSLSHSGF  
LSNLPXSIWQXSLLHWXISLHQQLSPLQISGKNGDXKNKeKKKKkkDNPYXYRLSFSVRRRTDSGTGXRIFR  
FPACLHNYSSEAGSNVSFXIISGVFMNNKRTITREQIKINGEIRERTATHIVGAHYETLCISGYIVEHNE  
MGEVIHNSEKLAEDLLPVTCPTCRVWIWYHTHEFTLDDFDTLSKGKDFVVTDLKELNIXKVHKXPALSNMLL  
SAGVSLRKIYMMSSRXLRIKLTMAFLSLARYPRIXRKTIFGSDQFPISVIFSREYPALNSSMAPTRTCPVHRY  
RPDCRLLSLTFPAIPQRRANAWFSELEDDSIPTSSRDTFDSFCNCVSNPSLNPAUTSILSMDLPRDSLLLVDGKY  
RFVWMFTGXKRKRCPKVSVSYSTVSINRLICXLHCGESXNRLLERLVLVRMTSRRXQDVSFCHFRSPVL  
ILRISTLRSRVSNPHTIIARSMSRLSSLLCDRRRIILRSDIRNGRAWPVTIFFSFSRARHTXTSAFTRLKSGK  
PRLLTLEHAIKXEMVAILTDPFSPLIXRSTSTAVTGKGVRPCLAHHADQTLVTLNLLAVLYRRNRNSLS  
ASRALMSSVREIATSLVLSVMGRSHVFLLILFLXPVV

The highlighted sequence is a predicted epitope that binds to human MHC class II (HLA-DR11) with a p value 1.15e-06. The prediction is based on PSSM alignment. As the Klebsiella pneumoniae NTUH-K2044 has plasmid and upon submission of this plasmid sequence it was found that a protein sequence could act as an epitope to MHC II (HLA-DR11) when enter the host with a p value of 1.15e-06. During the literature study of the organism it is noticed that there are many proteins in the organism

that could trigger an immune response in humans. So we have downloaded all those virulent sequences that could act as epitopes and submitted to the Vaxign. And the best region to be used as the vaccine against Klebsiella infections for the humans could be the putative outer membrane protein [Klebsiella pneumoniae NTUH-K2044] as it has a high binding affinity to the human MHC class I (HLA-B\*4001) with a p value 6.44e-06.

**Vaxign: Vaccine Target Prediction and Design**

**Sequence Alignment**

>664080 putative outer membrane protein [Klebsiella pneumoniae NTUH-K2044]  
MSRLFITFLLMACPTGSQAEALSREKIDGWLQHLGASDKFDASKGIDWGVMPGPFYTPELGLGIGTAVVG  
LYRPDPQDTTSQNSTLTSGYASSTGAFGLSVKNYTFDRDLWRVFDGSIANTPTYYWGQGFHAGDKDN  
EKEKYTAQVLTLRPTLYRQLIDHVYLGAGWSLAAQNADEMHDHDRPKIENTPQGPSVFSSGASIDINWDD  
RDFVPNPRRGQYANLRYTHYAPGLGSDTRFEFQLHYSHYHALSEKSVLAWEADGAFTQgevpwsmplLGS  
DERMRGYYQGRYRDKNVSGQLEYRRQLTWRHGIVAWAGAGTMGPSLSSLNNGRWLPSAGVGYRFEFK  
PRVNVRLDYGIGNGSSGFYFQVGEAF

*The highlighted sequence is a predicted epitope that binds to human MHC class I (HLA-B\*4001) with a p value 6.44e-06. The prediction is based on PSSM alignment.*

**Discussions:**

Klebsiella pneumoniae being a ubiquitous microbe is the major cause of the urinary infections was first explored by Friedlander after which Keh Ming et al

have sequenced the genome of Klebsiella pneumoniae NTUH-K2044. This microbe is known to contain 5.2Mbp which is used for its survival.

There are many treatments available for treating these infections. The major one is the usage of antibiotics and drugs. As with evolutionary progress this microbe has shown resistance to the antibiotics and drugs which has made the treatment to patients miserable as there is no available treatment to these diseases.

The only solution could be the use of vaccines. Vaccine is the part of the pathogen which triggers immune response in the host. Thus vaccine acts as the epitope to the host's immune system. Vaccine Informatics is an emerging research that focuses on the development and application of bioinformatics methods that can be used to facilitate every aspect of the preclinical, clinical vaccine enterprise. Many Immunoinformatics algorithms have been developed to predict T and B- cell immune epitopes for epitope vaccine development. This process of identification is called Reverse Vaccinology. By using this tool we have found that several regions can be used as vaccines for the human consumption, the regions like Mag A encoding genes can be used. And after the detailed study on the organism it was found that the genes like Mag A, rmp A, capsular serotypes and hypermucoviscosity were involved in the virulence of the strain. The putative outer membrane can be synthesized *in vivo* and can be used as a protein sub-unit protein against *Klebsiella* infections.

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