**International Journal of Advanced Biotechnology and Research(IJBR)**
ISSN 0976-2612, Online ISSN 2278–599X,
Vol6, Issue1, 2015, pp7-11
http://www.bipublication.com

# *In Silico* Characterization of Endotoxin: A Future Drug Target for *Neisseria meningitidis*

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[Received 01/12/2014, Accepted-20/12/2014]

## ABSTRACT:

*Neissaria maningitidis* is a gram negative bacterium, approximately 5000 people per year suffering from maningitidis disease out of that 21% are dying in India. Lipopolysaccharide is a component of the outermost membrane of *N. maningitidis*, which composed of hydrophobic domain known as lipid A, non-repeating core oligosaccharide and a distal polysaccharide O-antigen. Lipooligosaccharide and lipopolysaccharide have conserved inner cores composed of heptose and 3-deoxy-D-manno-octulosonic acids (kdo) which are anchored in outer membrane by lipid A, which acts as an endotoxin. Kdo transferase is an enzyme encoded by the kdtA gene, catalyzes the addition of kdo residues using cyclic monophosphate-kdo (cmp-kdo). The purpose of this investigation is to carry out *in silico* characterization of kdo transferase. Primary protein sequence analysis reveals that the pI value was 9.19, the total number of negatively charged residues (Asp+Glu) was 44 and the total number of positively charged residues (Arg+Lys) was 55. SOPMA was used to predict the secondary structure of protein which contains alpha helix 52.72%; extended strand 12.53% and random coil 27.66%. Homology modeling of kdo transferase was done using 2xci A as a template by SWISS MODEL and the model quality 92.7% was determined by PROCHECK. The functional domains contain KdtA and PRK05749 as a multi-domain and belong to Glycos_transf_N superfamily. Inhibition of kdo transferase which causes no addition of kdo residue to lipid A, this ultimately leads to the blocking of endotoxin pathway. Thus kdo transferase serves as a potential drug target for the treatment of maningitidis disease.

**Keywords**: Endotoxin, Drug target, *Neisseria meningitidis*, kdo transferase, kdtA gene

## [I] INTRODUCTION

*Neisseria meningitidis* is a gram-negative, aerobic, non endospore forming diplococcus belongs to the phylum Proteobacteria and cultures of the bacteria shows positive results for the enzyme cytochrome C oxidase test [13]. *Meningococcus* is a bacterium that can cause meningitis and other forms of meningococcal disease such as meningococcemia, a life-threatening sepsis. *N.*

*meningitidis* is a major cause of morbidity and mortality during childhood in industrialized countries and has been responsible for epidemics in Africa and in Asia [3]. It exists as normal flora (nonpathogenic) in the nasopharynx of up to 5–15% of adults. *Meningococcus* is only known infect humans and has never been isolated from animals; *Meningococcus* is spread through the exchange of saliva and other respiratory secretions during activities like coughing, sneezing, kissing, and chewing on toys. It initially produces general symptoms like fatigue and insomnia; it can rapidly progress from fever, headache and neck stiffness to coma and death. *N. meningitidis* is divided into 13 serogroups and example of this serological typing is A, B, C and W-135 [2]. The capsules are composed of Sialic acid (N- acetyl neuraminic acid, NANA) derivatives. The serogroup B capsule is composed of (α 2-8)-linked NANA [14]. The development of a vaccine against serogroup B poses the biggest problem due to the similarity between the B capsular polysaccharide structure and a polysialic acid containing glycopeptides that are a part of human brain tissue. Prevention of meningococcal disease will require the development of an effective vaccine to combat serogroup B, which is the cause of most meningococcal cases in developed countries [4].

The endotoxin of *N. Meningitidis* is structurally distinct from the Lipooligosaccharide of enteric gram negative bacteria. A major factor in the virulence of the organism is to release of outer-membrane vesicles that consist of lipooligosaccharide (Endotoxin), outer-membrane proteins, phospholipids and capsular polysaccharides [5]. Lipid A molecules of lipooligosaccharide (LOS) act as endotoxin and their effects are due to interaction with innate immune receptors. A necessary step in endotoxin biosynthesis is 3-deoxy-D-manno-octulosonic acid (Kdo) glycosylation of lipid A, catalyzed by the Kdo transferase KdtA (WaaA). In enteric gram-negative bacteria, this step is essential for survival. A nonpolar *kdtA*: *aphA-3* mutation was created in *Neisseria meningitidis* via allelic exchange, and the mutant was viable. Detailed structural analysis demonstrated that the endotoxin of the *kdtA*: *aphA-3* mutant was composed of fully acylated lipid A with variable phosphorylation but without Kdo glycosylation. revealed 35 enzymes of *N. Meningitides* that may be used as potential drug targets, as they belongs to pathways present only in the bacterium and not present in humans [5].

## [II] MATERIALS AND METHODS

### 2.1. Retrieval of KdtA sequence

KdtA protein sequence was retrieved from UniprotKB database according to the method of Michele Magrane, (2011). UniprotKB is the central hub for the collection of functional information of proteins [6].

### 2.2. Primary structure prediction

The primary structure was predicted using Protparam tool according to the method of Gasteiger [4]. Forphysic-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [11], Instability Index [12] Aliphatic Index [7] and Grand Average Hydropathy (GRAVY) [9] were computed using the Expasy Protparam server (http://expasy.org/cgi-bin/protpraram ).

### 2.3. Secondary structure prediction

The secondary structure was predicted using FASTA sequence of KdtA by SOPMA according to the method of Geourjon and Deleage [8]. It was employed for calculating the seconbil.ibcp.fr/cgi-bin/npsa_autodary structural features of the selected protein sequence.http://npsa-pmat.pl?page=/NPSA/ npsa_sopma.html

### 2.4. Homology Modeling

The protein sequence was subjected for comparative homology modeling via SWISS MODEL according to the method of Arnold [1] used to generate putative 3D model. SWISS-MODEL is fully automated protein structure homology modeling server to make the protein modeling accessible to all biotechnologist. The

SWISS MODEL performs the sequence alignments and searches for the putative template protein for generating the 3D model.

**2.5. Structure validation using PROCHECK**

According to the method of Laskowski [10] PROCHECK checks the stereo chemical quality of a protein structure, producing a number of PostScript plots analyzing its overall residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR. The structure was visualized and analyzed in Rasmol.

## [III] RESULTS

### 3.1. Protein primary sequence analysis

In this study primary structure of KdtA were predicted using Expasy's ProtParam server (http://expasy.org/cgi-bin/protparam ) according to that KdtA had 423 amino acid residues and molecular weight 47,272 Da. The maximum number of amino acids present in the sequence was found to be Alanine (11.6%) and least was that of Histidine (1.7%). The total number of negatively charged residues (Asp+Glu) was 44 and the total number of positively charged residues (Arg+Lys) was 55. The isoelectric point pI was 9.19. Protein is acidic in nature .The high aliphatic index (86.86) while instability index is 37.13. The ground average hydropathicity (GRAVY) is very low -0.232.

### 3.2. Secondary structure prediction of KdtA

The secondary structure is composed of alpha helix and beta sheets and is predicted by SOPMA as shown in [Table 1]. The secondary structure prediction was done and random coil was found to be 27.66% followed by extended strand 12.53% as shown in [Fig. 1].

The protein 3D structure was built using SWISS-MODEL and the template target alignment was done. The highest identity template 2xci.1 was selected to build the model as shown in [Fig. 2]. The model quality was checked using PROCHECK and the model quality was obtained about 92.7 % as shown in [Fig. 3]. The functional domains Glycos_ transf_N and Glycos_transf_1 were identified using Pfam as shown in [Fig. 4].

## [V] CONCLUSION

Kdo transferase is an enzyme encoded by the kdtA gene, catalyzes the addition of kdo residues using cyclic monophosphate-kdo (CMP-kdo). Blocking of kdo transferase enzyme which causes no addition of kdo residue to lipid A, this ultimately leads to the blocking of endotoxin pathway. Hence, blocking this enzyme serve as a potential drug target for treating *Neisseria meningitidis* disease.

| No | Secondary Structure | SOPMA |
|---|---|---|
| 1 | Alpha helix | 52.72% |
| 2 | B turns | 0.00% |
| 3 | Pi helix | 0.00% |
| 4 | Beta bridge | 0.00% |
| 5 | Extended strand | 12.53% |
| 6 | Beta turns | 7.09% |
| 7 | Bend region | 0.00% |
| 8 | Random coil | 27.66% |
| 9 | Ambiguous states | 0.00% |

**Table: 1.** Secondary structure prediction of KdtA by SOPMA

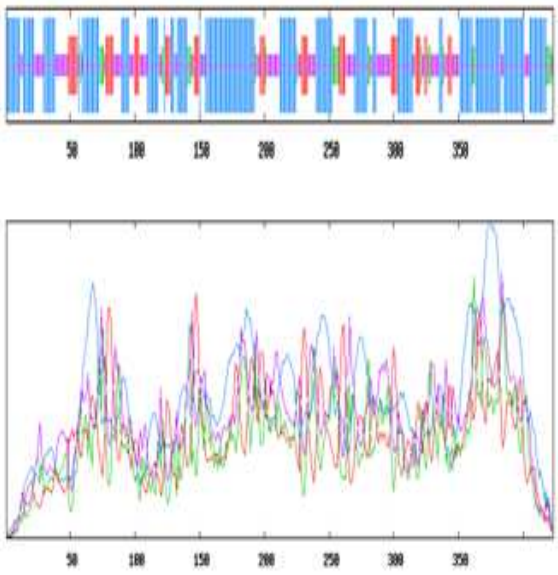


**Fig: 1.** Graphical representation of secondary element of kdtA protein.

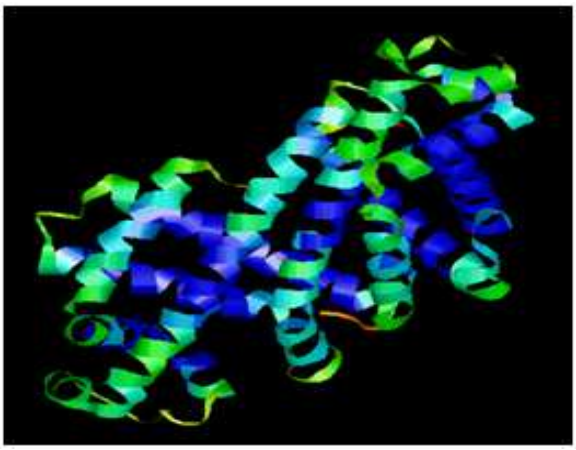

**Fig: 2.** Three dimensional structure of kdtA protein

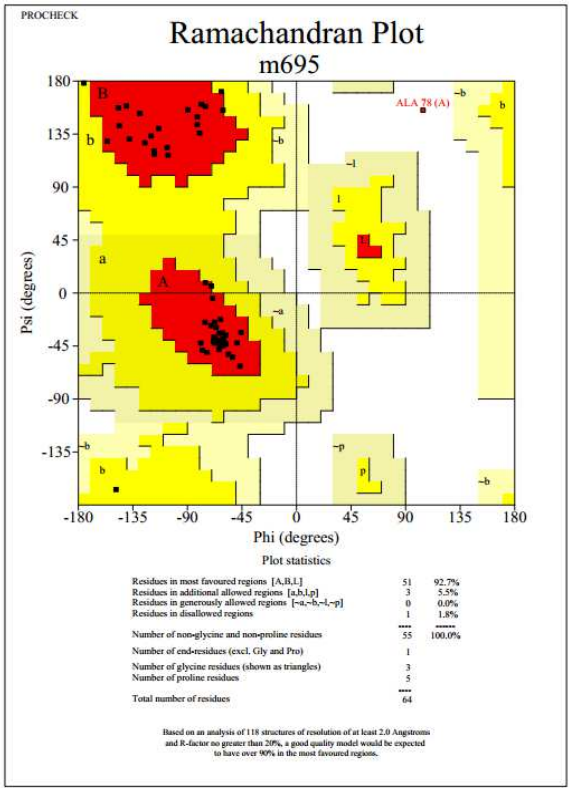


**Fig 3:** Graphical representation of kdtA Ramachandran Plot.

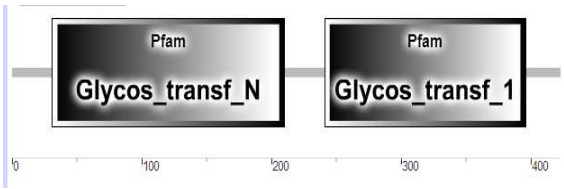


**Fig: 4.** conserved domains analysis of kdtA gene by Pfam.

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