Pak. J. Biochem. Mol. Biol., 2019, 52(1):37-51

Comparative modeling of potential drug targets of Neisseria meningitidis

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Abstract

Neisseria meningitidi is a gram negative proteobacterium which is the leading cause of sepsis and meningitis. There are five pathogenic serogroups (A, B, C, Y and W135) of *N. meningitidis.* Effective vaccines are available for all serogroups except serogroup B. Sequencing the genome of serogroup B strain MC58 gives efficient means of characterization of this pathogen. A library was built by in vitro modification of *N. meningitidis* DNA 73 genes were identified that are involved in pathogenesis out of which detailed structural bioinformatics was conducted on four cell envelope proteins (NMB1279, NMB1797,NMB0825 and NMB0713) using homology modeling studies. Due to high degree of sequence similarity, the three dimensional folds of all the targets were found to be similar with that of their respective templates. Binding sites/active sites residues almost showed strict conservation between target and template structures except for some residues which showed conservative substitution. Therefore, we believe that the overall three dimensional folds are of good quality and can be used for protein-ligand docking studies in order to find out potential lead compounds against *N. meningitidis* pathogenicity factors.

Keywords: *Neisseria meningitidis,* Homology modeling, NMB1279, NMB1797, NMB0825, NMB0713

Introduction

Neisseria meningitidis is a gram negative, proteobacterium and an encapsulated diplococcus which causes meningitidis and meningococcemia, a life-threatening sepsis [1]. *N. meningitidis* causes significant morbidity and mortality in children and young adults worldwide. It is classified into 12 serogroups according to the immunologic reactivity of their capsular polysaccharides, out of 12 only five of them (A, B, C, W135 & Y) are proved to be pathogenic [2-3]. Most cases of meningococcal disease are caused by serogroups A and C, for which polysaccharide vaccines are effective, and serogroup B, which is poorly immunogenic in humans [4].

In *N. meningitidis*, 73 genes were identified to be involved in this disease including 65 genes involved in bacteremic infection. These genes of unknown function were categorized into different

pathogenic groups, known virulence determinants, transport and binding proteins, amino acid biosynthesis, cell envelope, protein syntehsis etc [5].

Our aim was to perform structural bioinformatics study of potential drug targets which are related to cell envelope of *N. meningitidis*. Structural investigation of NMB0668 has already been carried out and published elsewhere [6]. During the present research, we have performed detailed structural analysis of four of the cell envelope proteins including NMB1279, NMB1797, NMB0825 and NMB0713 to find out possible binding modes of these potential antimicrobial targets by performing detailed active site analyses of the models. Whereas, NMB0345 protein did not show to have any suitable structural homologue in PDB (Table-1).

Table 1: List of selected templates for cell envelope protein involve in pathogenesis.

NMB	Template	Template Discription
1279	4ANR	Crystal structure of soluble lytic Transglycosylase SltB1 from *Pseudomonas aeruginosa*
1797	3A3D	Crystal structure of penicillin binding protein 4 (dacB) from *Haemophilus influenza*
0825	4E8W	Crystal Structure of *Burkholderia cenocepacia* HldA in Complex with competitive Inhibitor
0713	5VRG	Crystal Structural of Apolipoprotein N-acyltransferase from *E.Coli*

Materials and Methods

Homology modeling of *N.meningitis* cell envelope proteins

Homology modeling studies for pathogenic cell envelope protein of *N. meningitis* were performed. These proteins include: NMB0668, NMB1145, NMB1279, NMB1797, NMB0825 and NMB0713.

Secondary structure prediction

PSIpred is widely acknowledged as one of the most effective secondary structure predictors. PSIPRED were used for secondary structure prediction of NMB1279, NMB1797, NMB0825 and NMB0713 proteins.

Searching of best templates

Best templates for all of the target proteins were searched against PDB [7] using Psi-BLAST [8]. PDB entries having good similarity scores and having bound ligand, if possible, were chosen for comparative modeling. The selected templates for cell envelope proteins are listed in table-1.

Multiple sequence alignment

CLUSTALX [9] software was used for making multiple sequence alignments of target sequences with their respective homologues. Consequently, locally conserved regions were identified and the information was used to optimize pairwise sequence alignments between target proteins and their respective templates.

Homology model building and model evaluation

MODELLER (9v2) standalone software was used to perform homology modeling studies of target proteins based on target-template alignment.

The overall quality of comparative models was assessed using PROCHECK [10] and PROSA [11] standalone software. The variability between the Cα-backbones of 3D models and their templates was also analyzed in terms of root mean square deviation using the SUPERPOSE command of the MODELLER [12] software.

Protein modeling and structural analysis

Discovery Studio Visualizer and Visual Molecular Dynamics programs of protein structural analysis were used for analyzing the interactions between active/binding site residues and their ligands in addition to overall three dimensional analyses of target and template protein structures.

Results and Discussion

During the present study, we have focused on sequence analyses and structural bioinformatics of a group of four proteins which are involved in cell envelope of *N. meningitidis* and are known to involve in its pathogenesis.

Structural Elucidation of NMB1279 protein

NMB1279 is a putative membrane-bound lytic murein transglycosylase B from *N. meningitidis* serogroup B (strain MC58), which is involved in peptidoglycan hydrolysis or muropeptide recycling during cell division. Catalysis involves the cleavage of glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine residues in peptidoglycan. The best template for NMB1279 was found to be the crystal structure of soluble lytic transglycosylase from *P.aeruginosa* (PDB id: 4ANR). *N. meningitidis* transglycosylase showed 46% sequence similarity with transglycosylase protein from *P. aeruginosa* (Figure 2). According to the PSIPRED prediction, the protein is believed to have 17 alpha helices and 4 beta strands (Figure 1A). As transglycosylase B protein is known to be active in its monomeric form so we propose here the monomeric structure of *N. meningitidis* transglycosylase. *N. meningitidis* transglycosylase homology model showed rmsd of 0.47 Å with *P. aeruginosa* lytic transglycosylase. Three dimensional structure of NMB1279 protein model showed that the overall fold is mainly alpha helical and have three major domains: an N-terminal alpha domain, a core domain and a C-terminal beta-sheet domain. The overall three dimensional structure of *N. meningitidis* transglycosylase and *P. aeruginosa* lytic transglycosylase was found to be similar (Figure 3A). All the active site residues including Glu135 that played an important role in catalysis [13] were found to be conserved in *N. meningitidis* lytic transglycosylase model thus *N. meningitidis* lytic transglycosylase may have similar activity as that of *P. aeruginosa*lytic transglycosylase. Only one residue i.e. Tyr270 has been substituted by Trp270 in *N. meningitidis* lytic transglycosylase model (Figure 3B). The PROSA plot showed good energy profile with 3.81 Z-score. Ramachandran plot showed no residues in the disallowed regions and therefore the model was thought to have good stereochemistry (Figure 3C & 3D).

Structural elucidation of NMB1797 Protein

NMB1797 is a Penicillin-binding protein 3 from *N. meningitidis*serogroup B (strain MC58). The penicillin-binding proteins are responsible to synthesize and remodel the cell wall peptidoglycan. Penicillin binding protein 4 from *Haemophilus influenzae* having PDB id: 3A3D was considered to be best structural homologue to which *N. meningitidis* Penicillin-binding protein 3 showed 29% sequence similarity (Figure 4). According to the prediction, the protein was believed to have 12 alpha helices and 21 beta strands (Figure 1D). Homology model of *N.*

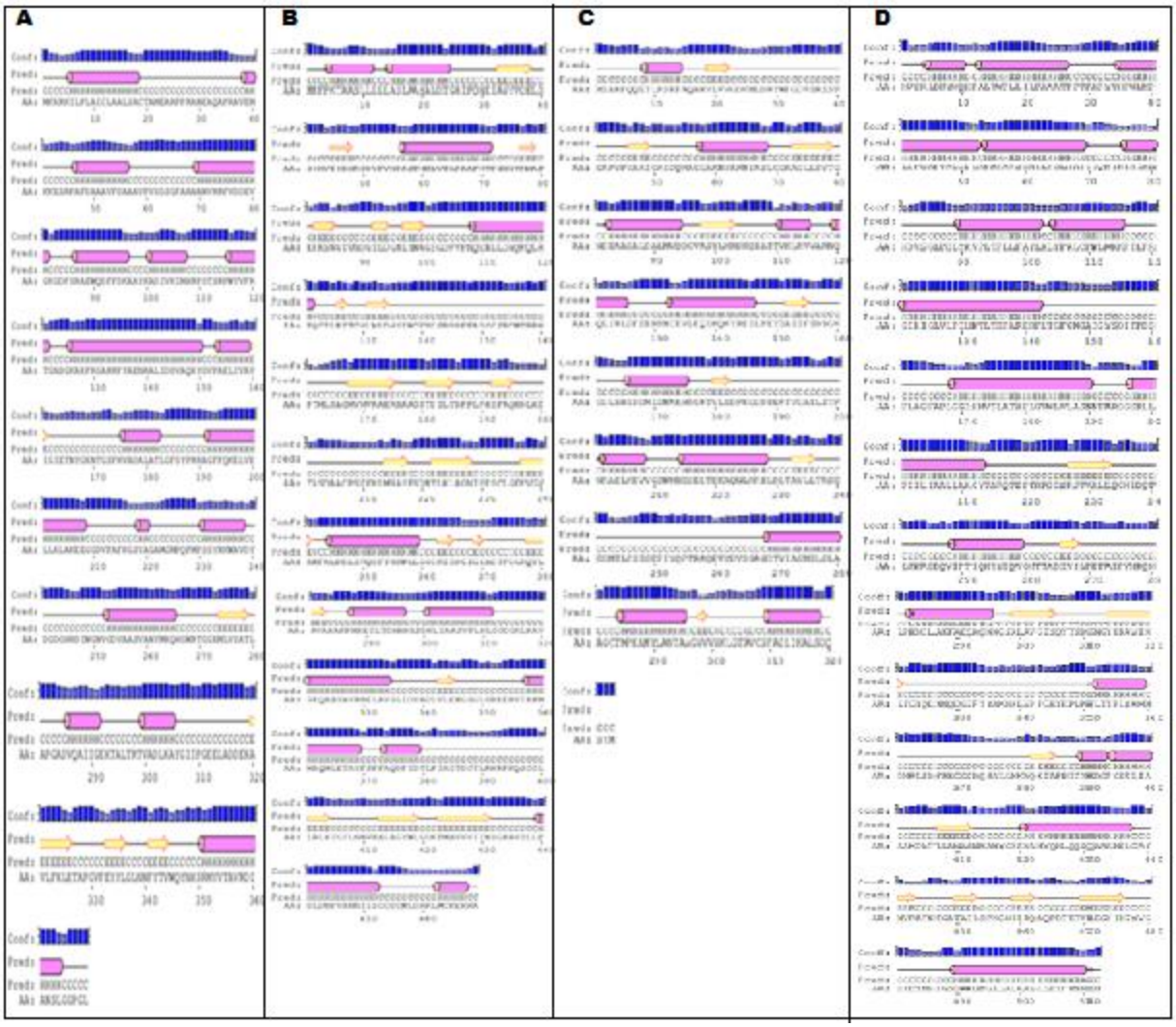

Figure 1: Secondary structure prediction results of NMB1279 (A), NMB1797 (B), NMB0825 (C) and NMB0713 (D) **proteins.**

meningitidis penicillin binding protein was constructed using crystal structure coordinates of *H. influenzae*as a template. Three-dimensional model of *N. meningitidis* penicillin binding protein 3 was found to consist of three domains which were found to be similar with penicillin binding protein 4 from *H. influenzae.* The overall fold of the model was found to be similar with penicillin binding protein of *H. influenzae* (Figure 5A). The superposed structures of *N.meningitidis* penicillin binding protein 3 and *H. influenzae* penicillin binding protein 4 were shown to have an rmsd of 0.7628 Å. The binding site of penicillin binding protein 4 of *H. influenzae* was found in an open groove on the protein surface surrounded by three main motifs i.e. KTG, SXXK and SXN. Residues that were involved in binding include Lys420, Thr421, G422, Ser69, Thr70, Gln71,

Lys72 and Ser310. All the residues were found to be conserved except Met71 which has been substituted with Gln71 in *N.meningitis* [14]. The active site residue conservation *N. meningitidis* model and *H. influenza* crystal structure suggesting that *N. meningitis* penicillin binding protein 3 has similar activity like *H. influenzae* penicillin binding protein 4 (Figure 5B). The homology model of *N. meningitidis* penicillin binding protein 3 was found to be of good stereochemistry and good energy profile (Figure 5B & 5C).

Structural elucidation of NMB0825 Protein

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Sec.str.pred αααααααααααα     αααααααααα    αααααααα          ααααααα    αααα
NMB1279   1  NVRRFVDDEVGKGDFSRAEWQDFFDKAAYKADIVKIMHRPSTS-RPWYVFRTGNSGKAKF 59
4ANR     24  QVAEFVSEMTRDYGFAGEQLMGLFRDVNRKQSILDAISRPAERVKQWKEYRPIFISDARI 83
Sec.str.pred αααααααααααααα   αααααααααα    αααααααα         αααααααααα ααααα

Sec.str.pred αααααααααααααααααααααα   αααααααββ               αααααααα
NMB1279  60  RGARRFYAENRALIDDVAQKYGVPAELIVAVIGIETNYGKNTGSFRVADALATLGFDYPR 119
4ANR     84  SRGVDFWNKHAEDLARAEKEYGVPAEIIVSIIGVETFFGRNTGSYRVMDALSTLGFDYPP 143
Sec.str.pred αααααααααα ααααααααααα   αααααααααααα        ββαααααααααααα αα

Sec.str.pred   αααααααααααααααααα          αα          αααααααα
NMB1279  120 RAGFFQKELVELLKLAKEEGGDVFAFKGSYAGAMGMPQFMPSSYRKWAVDYDGDGHRDIW 159
4ANR     144 RADFFRKELRERFLLLAREQQVDPLSLTGSYAGAMGLPQFMPSSFRAYAVDFDGDGHINIW 203
Sec.str.pred αααααααααααααααααααα   ααα ββ            αααααααα

Sec.str.pred    αααααααααααααα        βββββββ      αααααα        ααααααα
NMB1279  160 GNVGDVAASVANYMKQHGWRTGGKMLVSATLAPGADVQAIIGEKTALTRTVADLKAYGII 219
4ANR     204 SDPTDAIGSVASYFKQHGWVT-GEPVVSVAEINDESAESAVTRGVDPTMSLGELRARGWR 262
Sec.str.pred   αααααααααααααα          βββββββ  ααααααα        ββαααααα  ββ

Sec.str.pred        βββββββ      ββββ    ββββ      αααααααααααααα
NMB1279  220 PLADDEKAVLFKLETAPGVFEYYLGLNNFYTVWQYNHSRMYVTAVRDIANSL 272
4ANR     263 TLRDDQKVTAMRFVGDKGI-EYWVGLPNFYVITRYNRSAMYAMAVYQLAGEI 316
Sec.str.pred       βββββββββ     βββββ αααααααααα   αααααααααααααα
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Figure 2: Structure based pairwise sequence alignment of *N. meningitis and P. Aeruginosa* Lytic Transglycosylases. The alpha helices are shown by "α" and beta sheets are shown by "β". Active site residue was written in bold and underlined.

NMB0825 is a putative ADP-heptose synthase from *N. meningitidis* serogroup B (strain MC58). Crystal Structure Of *Burkholderia Cenocepacia* D-Glycero-β-D-manno-heptose-7-Phosphate Kinase (HldA), having PDB id 4E8W, was considered to be best structural homologue. *N. meningitidis* ADP-heptose synthase protein sequence showed 57% sequence similarity with

Burkholderia Cenocepacia D-Glycero-β-D-manno-heptose-7-Phosphate Kinase (HldA) protein sequence (Figure 6).

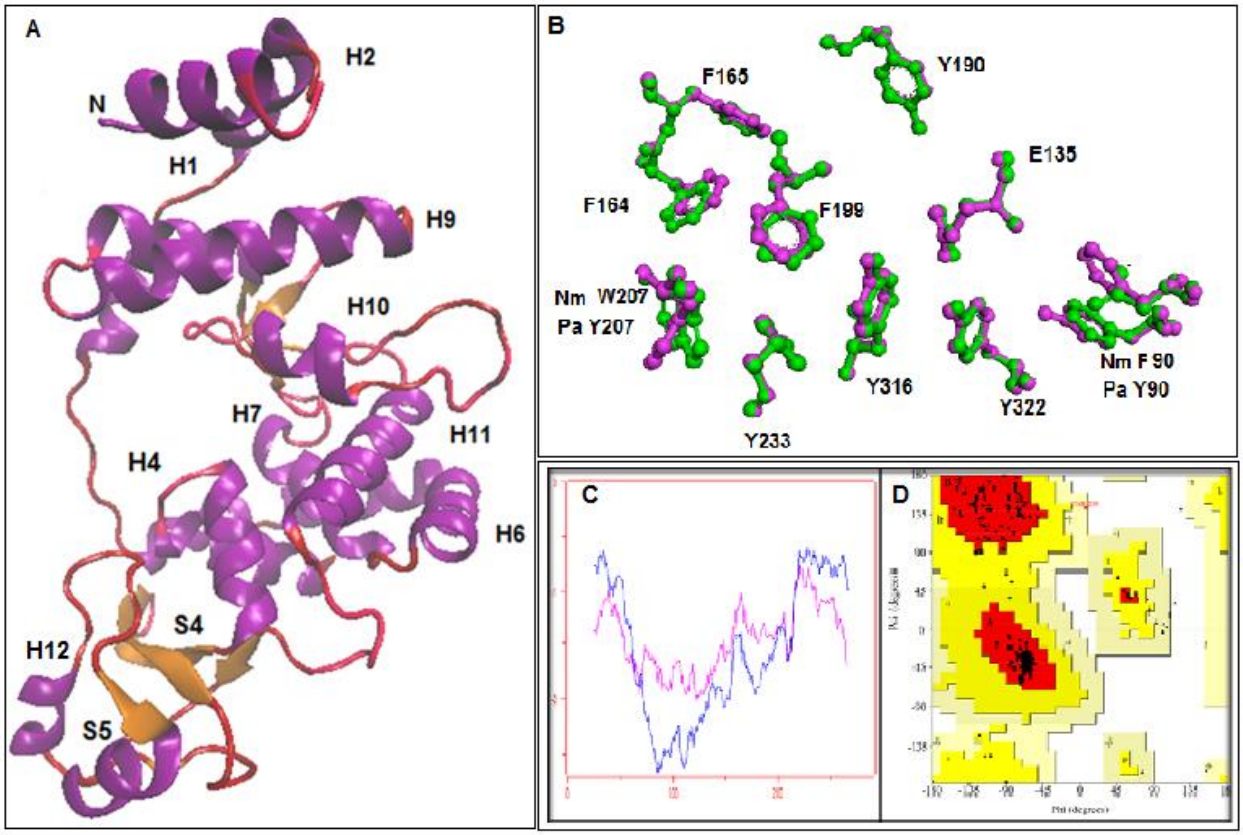

Figure 3: Three dimensional structure of NMB1279 protein model (A); Comparative analysis of active site residues (B); PROSA plot (C) and Ramachandran plot (D).

According to the secondary structure prediction results, the protein was believed to have 12 alpha helices and 8 beta strands (Figure 1C). Homology model of *N. meningitidis* ADP-heptose synthase was constructed using *Burkholderia Cenocepacia* HldA protein structure (PDB id; 4E8W) as a template. The superposed structures of *N. meningitidis* ADP-heptose synthase and *Burkholderia Cenocepacia* HldA were shown to have an rmsd of 0.5Å. Three dimensional structure of NMB0825 model showed that the protein consists of two protomers. Each protomer has α/β core and twisted beta sheets. The overall three-dimensional structure of *N. meningitidis* was found to be simillar with *B. Cenocepacia* protein [15] (Figure 7A). Model of *N. meningitidis* ADP-heptose synthase was evaluated and the calculated z-score was -4.18 showing good energy profile of the

constructed model (Figure 7B). Acive sites residues of *B. Cenocepacia* HldA protein are: A257, V265, G267 and V301 [15]. All of these residues were found to be conserved in *N. meningitidis* ADP-heptose synthase model, suggesting that the *N. meningitidis* ADP-heptose synthase has the same activity with that of *B. Cenocepacia* HldA protein (Figure 7C).

Figure 4: Structure based pairwise sequence alignment of *N. meningitis and H. influenza* penicillin binding proteins. The alpha helices are shown by "α" and beta sheets are shown by "β", active site residues are written in bold and underlined.

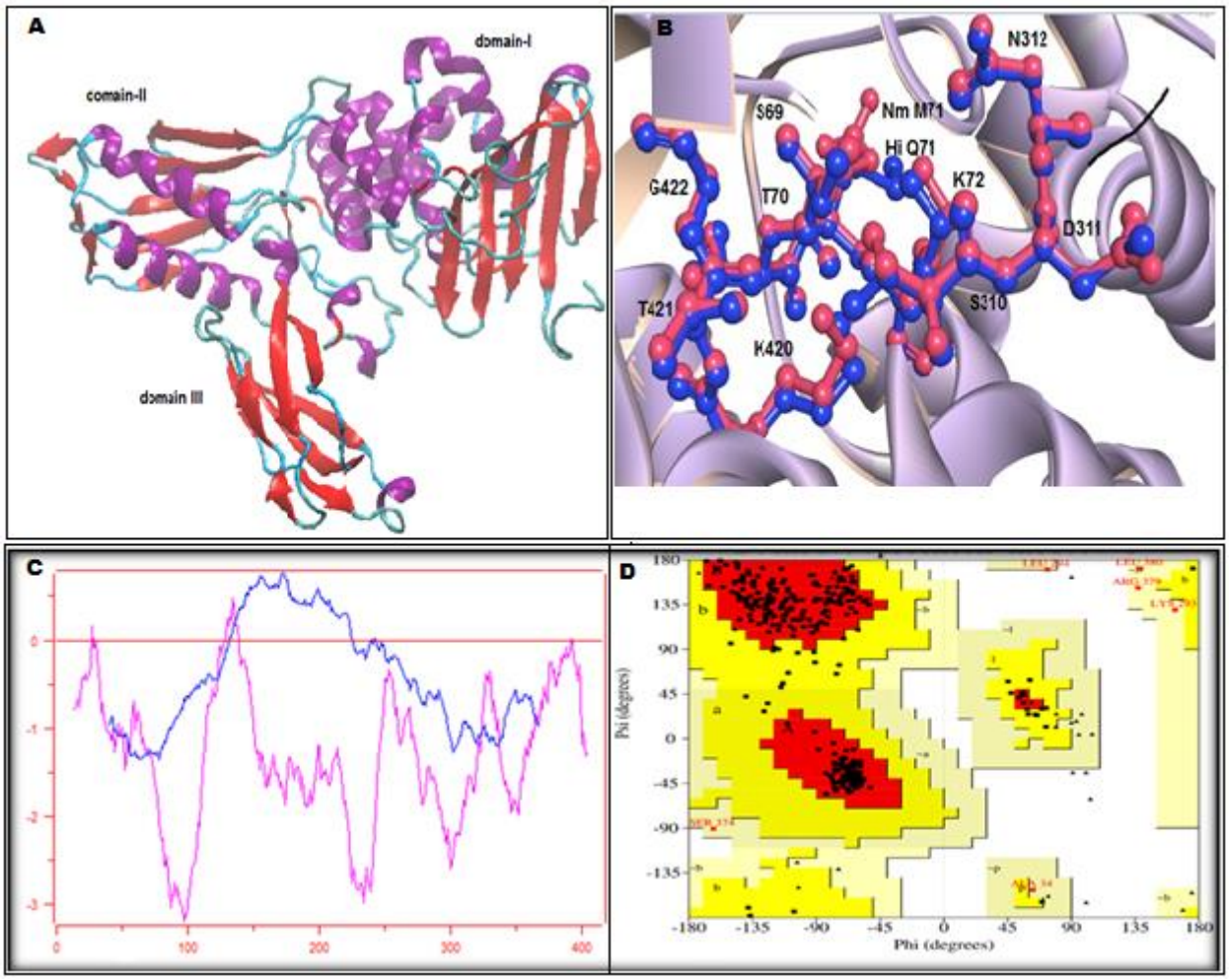

Figure 5: Homology model of *N. meningitidis* penicillin binding protein (A); **C**omparative analysis of active site residues from superposed structure of *N. meningitidis* penicillin binding protein 3 (blue) and *H.influenzae* penicillin binding protein 4 (red)(B); Evaluation of penicillin binding protein model by PROSA plot (C) and Ramachandran plot (D).

Structural elucidation of NMB0713 protein

NMB0713 is an Apolipoprotein N-acyltransferase from *N. meningitidis* serogroup B MC58.It transfers the fatty acyl group on membrane lipoproteins.BLAST results showed 14 hits, out of them *Escherichia coli* Apolipoprotein N-acyltransferase having PDB id 5VRG was considered to be best structural homolologue having 31% sequence similarity (Figure 8). According to secondary structure prediction, *N. meningitidis* Apolipoprotein N-acyltransferase is composed of 17 alpha helices and 10 beta strands (Figure 1D). Protein homology modeling of NMB0713 was carried out using crystal coordinates of *E.coli* apolipoprotein N-acyltransferase as template. The superposed

structures of *N. meningitidis* apolipoprotein N-acyltransferase and *E.coli* apolipoprotein N-acyltransferase was shown to have an rmsd of 0.9610Å. Three-dimensional model of *N. meningitidis* apolipoprotein N-acyltransferase shown to have an eight transmembrane helices-fold and a cytoplasmic domain. The overall architecture of *N. meningitidis* apolipoprotein N-acyltransferase model was found to be similar with apolipoprotein N-acyltransferase from *E.coli* [16] *(*Figure 9A). Amino acid residues of *E.coli* apolipoprotein N-acyltransferase involved in catalysis includes K335, E257 and C287 , all these was found to be conserved in *N.meningitidis.* apolipoprotein N-acyltransferase model, suggesting that both proteins have similar catalytic strategies (Figure 9B). The model had a good energy profile (Figure 9C).

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Sec.str.pred          ββββ                          ββββ         αααααααααα
NMB0825     1 KSRFAQAKVLVVGDVMLDRYWFGDVSRISPEAPVPVAKIGRIDQRAGGAANVARNIASLG 60
4e8w       48 REQLARSRVLVVGDVMLDRYWFGNVDRISPEAPVPVVHVQRQEERLGGAANVARNAVTLG 107
Sec.str.pred     ααα ββββββ ββββββββββ             βββββββββββαααααααααα


Sec.str.pred  βββββββββ   αααααααααααααα     βββ       ααααα      ααα
NMB0825    61 GRAGLLSVTGNDEAADALDALMVQDGVASYLMRDKQIATTVKLRVVARNQQLIRLDFEEH 120
4e8w      108 GQAGLLCVVGCDEPGERIVELLGSSGVTPHLERDPALPTTIKLRVLARQQQLLRVDFEAM 167
Sec.str.pred  βββββββββ   αααααααααααα   βββββββββ      βββββββ   βββββββββ


Sec.str.pred  ααααααααααααααα    ββββ          αααααααααααα
NMB0825 121 PNCEVLEQIKQKYREILPEYDAIIFSDYGKGGLSHISDMIDWAKHAGKTVLIDPKGDDYE  180
4e8w   168  PTHEVLLAGLARFDVLLPQHDVVLMSDYAKGGLTHVTTMIEKARAAGKAVLVDPKGDDWA  227
Sec.str.pred  ααααααααααααααααα  ββββ          ααααααααααα   ββββ       α


Sec.str.pred           αααααα       ααααααααααααα    ββββ       βββ
NMB0825 181 KYVGATLITPNRAELKEVVGSWKNESELTEKAQNLRRHLDLTAVLLTRSEEGMTLFSEGE  240
4e8w   228  RYRGASLITPNRAELREVVGQWKSEDDLRARVANLRAELDIDALLLTRSEEGMTLFSAGG  287
Sec.str.pred           αααααααα     ααααααααααααα    βββββββαα ββββββββββ

Sec.str.prd                  ααααααααααααα    ααααααααααα   ββ         α
NMB0825 241 PIYQPTRAQEVYDVSGAGDTVIAGMGLGLAAGCTMPEAMYLANTAAGVVVAK3GTAVCSF 300
4e8w   288  ELHAPALAREVFDVSGAGDTVIATVATMLGAGVPLVDAVVLANRAAGIVVGKLGTATVDY
Sec.str.prd ββββ           ααααααααααααααα    ααααααααααααα           α

Sec.str.prd αααα
NMB0825 301 AELI  305
4e8w   348  DELF  352
Sec.str.prd αααα
```

Figure 6: Structure based pairwise sequence alignment of *N. meningitis* and *B. Cenocepacia ADP-heptose synthases*. The alpha helices are shown by "α" and beta sheets are shown by "β", active site residues are written in bold and underlined.

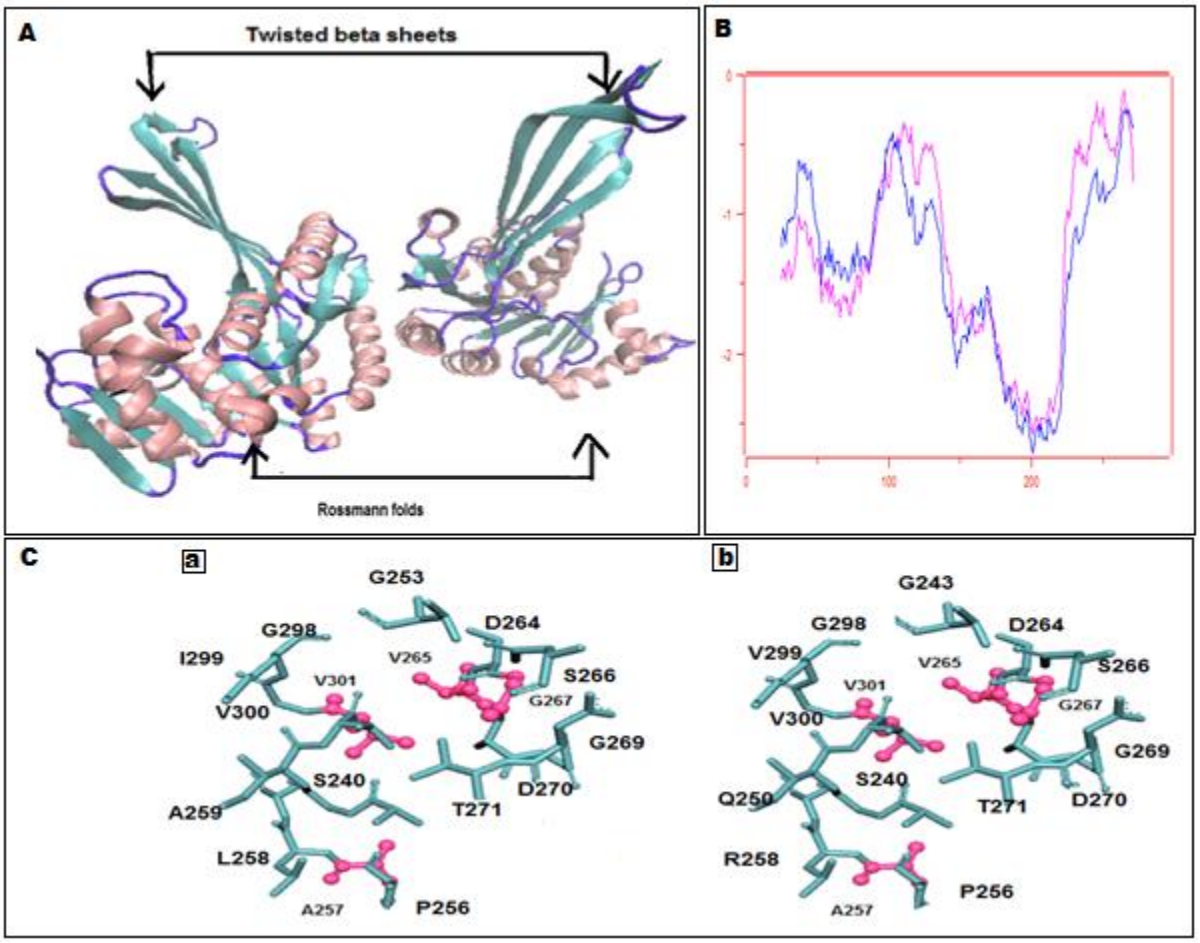

Figure 7: Homology model of *N. meningitidis* ADP-heptose synthase (A), PROSA plot of *N. meningitidis* ADP-heptose synthase model (blue) and *B. Cenocepacia* crystal structure (magenta) (B) and Comparision of active site residues of (C-a*) B. Cenocepacia* and (C-b) *N. meningitidis*. The residues involved in catalysis are shown by pink color.

The present study revealed that the three dimensional folds of all the pathogenicity factors involved in cell envelope metabolism were found to be similar with their respective templates. Binding sites/active sites residues almost showed strict conservation between target and template structures except for some residues which showed conservative substitution. Therefore, we believe that the overall three dimensional folds are of good quality and can be used for protein-ligand docking studies in order to find out potential lead compounds against *N. meningitidis* pathogenicity factors.

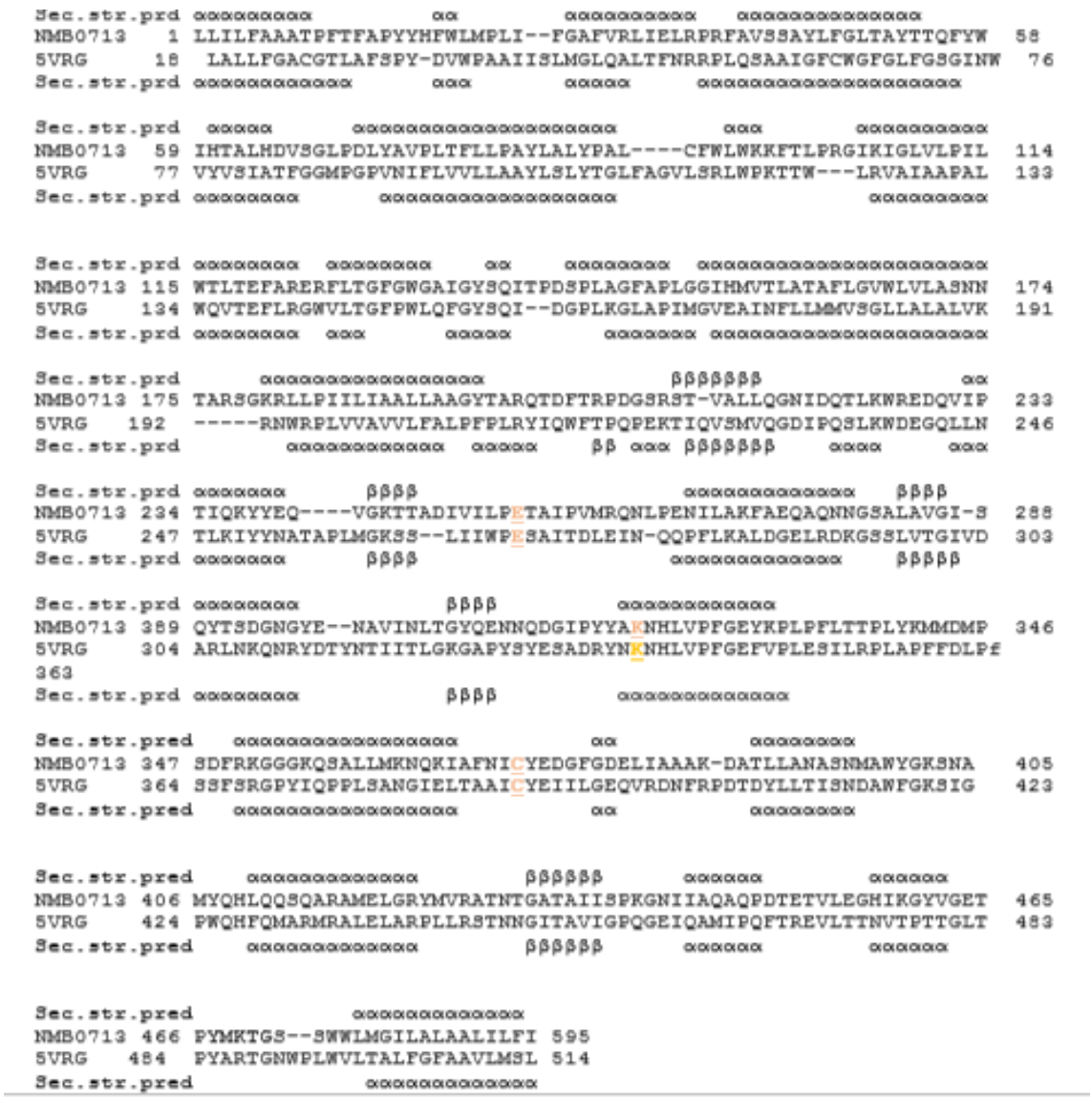

Figure 8: Structure based pairwise sequence alignment of *N. meningitis* Apolipoprotein N-acyltransferase and *E. coli* Apolipoprotein N-acyltransferase. The alpha helices are shown by "α" and beta sheets are shown by "β", active site residues are written in bold and underlined.

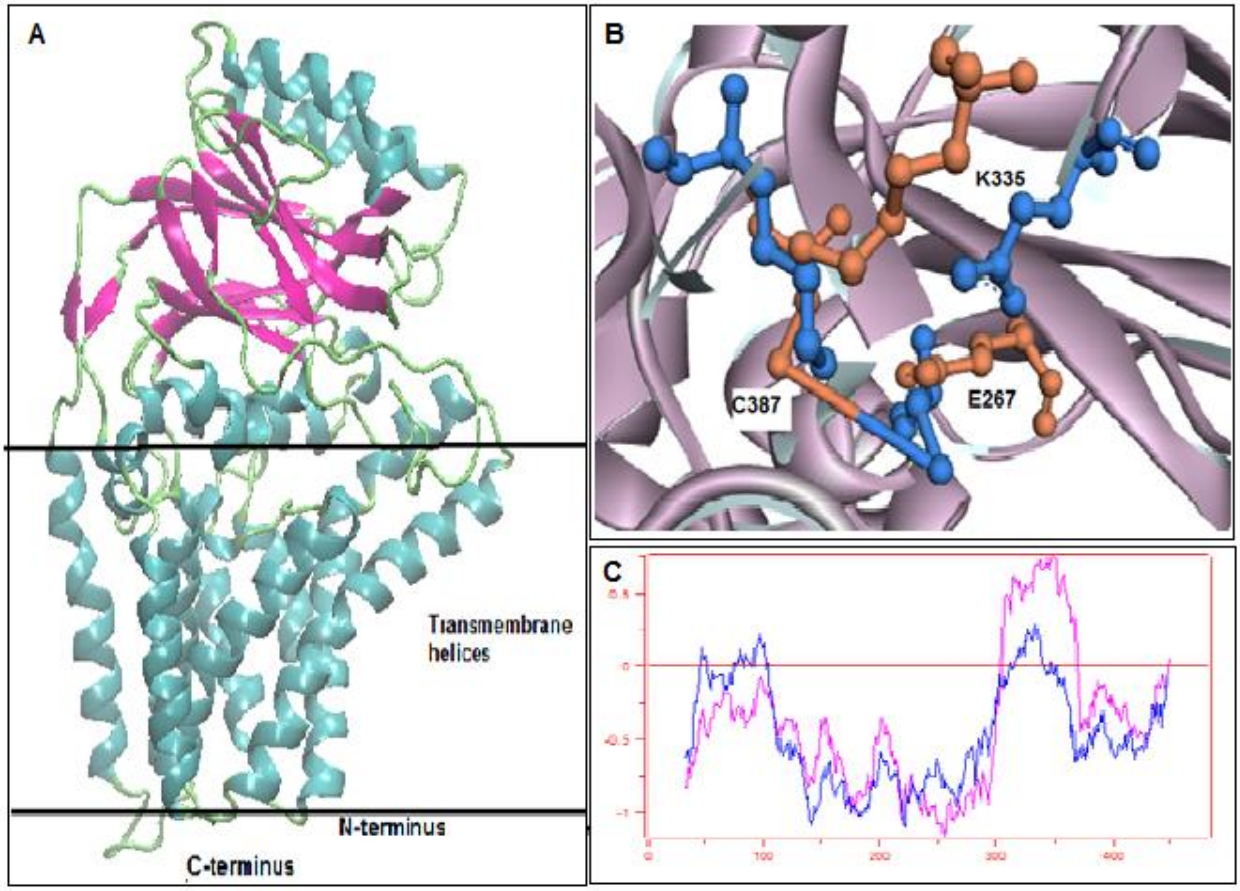

Figure 9: Homology model of *N. meningitidis* Apolipoprotein N-acyltransferase (A); Comparative analysis of active site residues from superposed structures of *N. meningitidis* (orange) and *E.coli* (blue) (B) and PROSA plot of *N. meningitidis* (blue) and *E.coli* (magenta) (C).

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