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In-silico three dimensional structure prediction of important *Neisseria meningitidis* proteins

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Abstract: Pathogenic bacteria *Neisseria meningitidis* cause serious infection i.e. meningitis (infection of the brain) worldwide. Among five pathogenic serogroups, serogroup B causes life threatening illness as there is no effective vaccine available due to its poor immunogenicity. A total of 73 genes in *N. meningitidis* genome have identified that were proved to be essential for meningococcal disease and were considered as crucial drug targets. We targeted five of those proteins, which are known to involve in amino acid biosynthesis, for homology-based three dimensional structure determinations by MODELLER (v9.19) and evaluated the models by PROSA and PROCHECK programs. Detailed structural analyses of NMB0358, NMB0943, NMB1446, NMB1577 and NMB1814 proteins were carried out during the present research. Based on a high degree of sequence conservation between target and template protein sequences, excellent models were built. The overall three dimensional architectures as well as topologies of all the proteins were quite similar with that of the templates. Active site residues of all the homology models were quite conserved with respect to their respective templates indicating similar catalytic mechanisms in these orthologues. Here, we are reporting, for the first time, detailed three dimensional folds of *N. meningitidis* pathogenic factors involved in a crucial cellular metabolic pathway. Moreover, the three dimensional structural information of these important drug targets would be utilized in computer-aided drug designing in future.

Keywords: *Neisseria meningitidis*, homology modeling, amino acid biosynthesis, MODELLER.

INTRODUCTION

Bacterial growth phase can be interrupted by preventing the biosynthesis and assembly of crucial components involved in bacterial processes and it has been established as a successful approach for the development of new antibacterial drugs (Walsh, 2003; Clatworthy *et al*., 2007). Hence a detailed understanding of the overall fold and active site architecture involving catalytic mechanism of selected enzymes can be helpful for the rational drug designing against deadly pathogens.

Subject of our study is a deadly disease known as meningococcal meningitis with 1.2 million reported cases globally. The pathogen *Neisseria meningitidis* is rampant and invasive, colonizing the upper respiratory tract of 10% of healthy individuals in non-epidemic settings and causing 135 thousand annual deaths, despite intense antibiotic treatment (Afrough *et al*., 2018; Yazdankhah, 2004; Vacca *et al*., 2019). The present work is focused on the serogroup B (strain MC58). A smaller number of Serogroup B outbreaks have been observed worldwide but serogroup B outbreaks are usually prolonged, cause substantial morbidity and mortality (Swartley *et al*., 1997; Tzeng & Stephens, 2000; Rosenstein *et al*., 1999; Dyet & Martin, 2006) and accounts for 50% of all meningococcal disease (Caesar et. al., 2013). This is because serogroup B is non-immunogenic in humans, therefore developing effective vaccine against this particular serogroup became challenging. The reason for its non-immunogenicity is the similarity of capsule polysaccharide that is uniquely composed of polysialic acid, with glycoproteins on the surface of human neurological tissue (Finne *et al*., 1983; Nair, 2012). Therefore, polysaccharide vaccines that had been effective against other serogroups cannot be employed against Serogroup B (Griffiss *et al*., 1991). This gap necessitates the exploration of proteins involved in pathogenesis and their active sites that may be used as drug targets. Serogroup B strain MC58, isolated from a case of invasive infection, was sequenced and opened windows to the molecular characterization of this pathogen. The genome consisted of 2,272,351 total base pairs. 2158 coding regions were predicted out of which 1158 (53.7%) were found to have biological role. Whereas, Number of protein coding regions that remained uncharacterized and designated as hypothetical was 1000 (46%) (Swartley *et al*., 1997). To further understand its pathogenesis, a library of 2,850 insertional mutants of *N. meningitidis* was built as par their capacity to cause systemic infection in an infant rat model. The study identified 73 genes involved in bacteremic disease. Eight insertions were in genes already known to encode pathogenicity factors while remaining 65 genes' involvement in bacteremic infection had not been described previously. The new genes identified in the study may help elucidate pathogenic mechanisms leading to the development of new vaccines and drug targets. The genes were categorized according to the different pathogenic groups i.e. Known Virulence Determinants;

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Transport And Binding Proteins; Amino Acid Biosynthesis; Cell Envelope; Protein Synthesis; Transcription; Energy Metabolism; Regulatory Functions; Cellular Processes; Biosynthesis Of Cofactors; Cell Division; Fatty Acid And Phospholipid Metabolism etc (Sun *et al*., 2000).

The aim of the present study was to determine the overall three dimensional folds of selected *N. meningitidis* enzymes involved in its amino acid biosynthesis including their active site architectures using homology modeling studies. The provision of three dimensional structural information of these important drug targets will be helpful in designing novel drug molecules against *N. Meningitidis* through computer aided drug designing.

MATERIALS AND METHODS

Homology modeling studies

In Homology modeling, a three-dimensional model of a protein with unknown structure is built on sequence similarity basis (Blundell *et al*., 1987; Sali, 1995). We selected *N. meningitidis* amino acid biosynthetic proteins including NMB0358, NMB0943, NMB1446, NMB1577 and NMB1814 and gene products and built their structural models using homology modeling methods.

Pre modeling secondary structure prediction

Secondary Structure prediction (Jones, 1999) helps in establishing alignments especially for identifying related secondary structural features, when the positioning of insertions and deletions is ambiguous. We mainly used the PSIPRED secondary structure prediction method, (Buchan *et al*., 2013).

Template search

In our study, NCBI variant Position Specific Iterated (PSI) BLAST (Stephen *et al*., 1997) was used to search homologs against PDB (Altschul *et al*., 1997; Berman *et al*., 2002). PDB structures showing best similarity with the corresponding target proteins were selected as the templates for homology modeling. Homologs complexed with substrate/inhibitor were preferred.

Model building, refinement and evaluation

The program MODELLER (9v2) (Sali & Blundell, 1993) was used to construct three dimensional structural models of target proteins using crystal or solution structure atomic coordinates of templates on the basis of target-template alignments. The reliability of the model was tested by the programs PROCHECK (Laskowski *et al.,* 1993) and ProSA (Sippl, 1995). PROCHECK provides a detailed check on the stereochemistry of all atoms in a given model of protein structure. However, ProSA provides a more stringent test of the overall fold and side-chain packing of the homology models by calculating energy profile and a Z-score.

Protein visualization and structural analysis

These analyses were carried out using DS Visualizer by Accelrys Inc.

RESULTS

Structural studies of shikimate dehydrogenase

N. meningitidis shikimate dehydrogenase catalyzes the reversible NADPH linked reduction of 3-dehydroshikimate (DHSA) to yield shikimate (Herrmann & Weaver, 1999). NCBI PSI-BLAST technique returned the several full length templates with significant identities. Chain A of Shikimate Dehydrogenase (AroE) complexed with NADP+ having PDB id 1NYT (Michel *et al*., 2003) was selected as the best template. The query coverage and sequence identity were 97% and 52%, respectively. From the PSIPRED, the structure seems predominately composed of Alpha helix with high confidence interval (fig. 1). The model has an overall good stereochemistry (fig. 2) and excellent energy profile (fig. 3A). The overall architecture of the model suggested a somewhat elongated shape and just like the template, appeared to have two domains. Both domains have alpha/beta architectures connected by short linkers. The arrangement of these two domains along the connecting helices creates a deep groove in which the cofactor NADP+ (or NAD+) binds (fig. 4A). The active site residues of the model were well conserved (fig. 4B). From the overall conservation pattern, we can infer the conservation of function as well.

Structural studies of 5, 10-methylenetetrahydrofolate reductase

N. meningitidis 5, 10-methylenetetrahydrofolate reductase enzyme is responsible to catalyze the reduction of 5-methyltetrahydrofolate into 5, 10-methylene tetrahydrofolate which is the only methyl donor to homocysteine in the production of methionine (Jacques *et al*., 1996). *E. coli* methylenetetrahydrofolate reductase complexed with its substrate i.e., methyltetrahydrofolate having PDB id 3FSU (Lee *et al*, 2009), was chosen as a suitable template. The query coverage and identity were 99% and 73%, respectively. From the PSIPRED, the structure seems predominately composed of Alpha helix with high confidence interval (fig. 1). The overall architecture of the model represented a barrel structure having alpha and beta elements in an alternative pattern (fig. 5A). The substrate was located in the core. Analysis of overall model quality through ProSA revealed all residues to be well below zero and a Z score of -3.62 (fig. 3B). Procheck on the other hand calculated most of the residues to be in favored or allowed region of Ramachandran plot (fig. 2). The level of active site residue conservation between *N. meningitidis* and *E. coli* 5, 10-methylenetetrahydrofolate reductases (fig. 5B) was as high as 100% suggesting same structure as well as same function for the two enzymes.

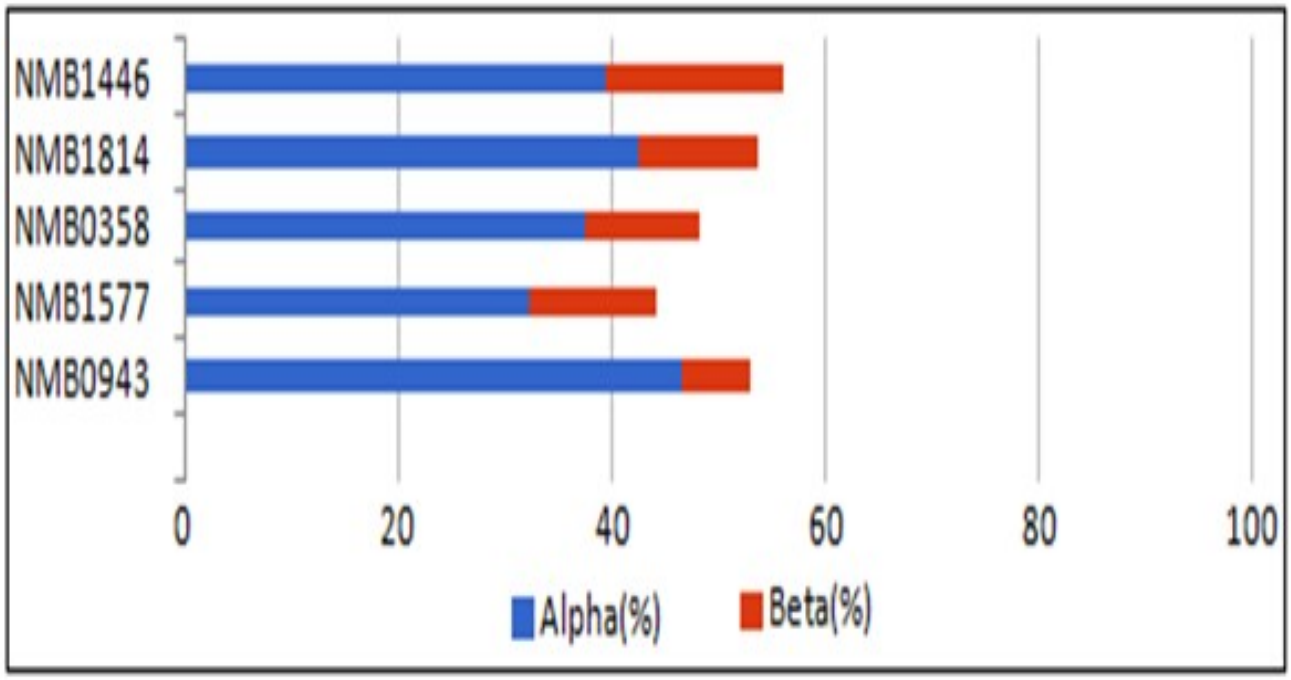

Fig. 1: Secondary structure prediction results by PsiPred.

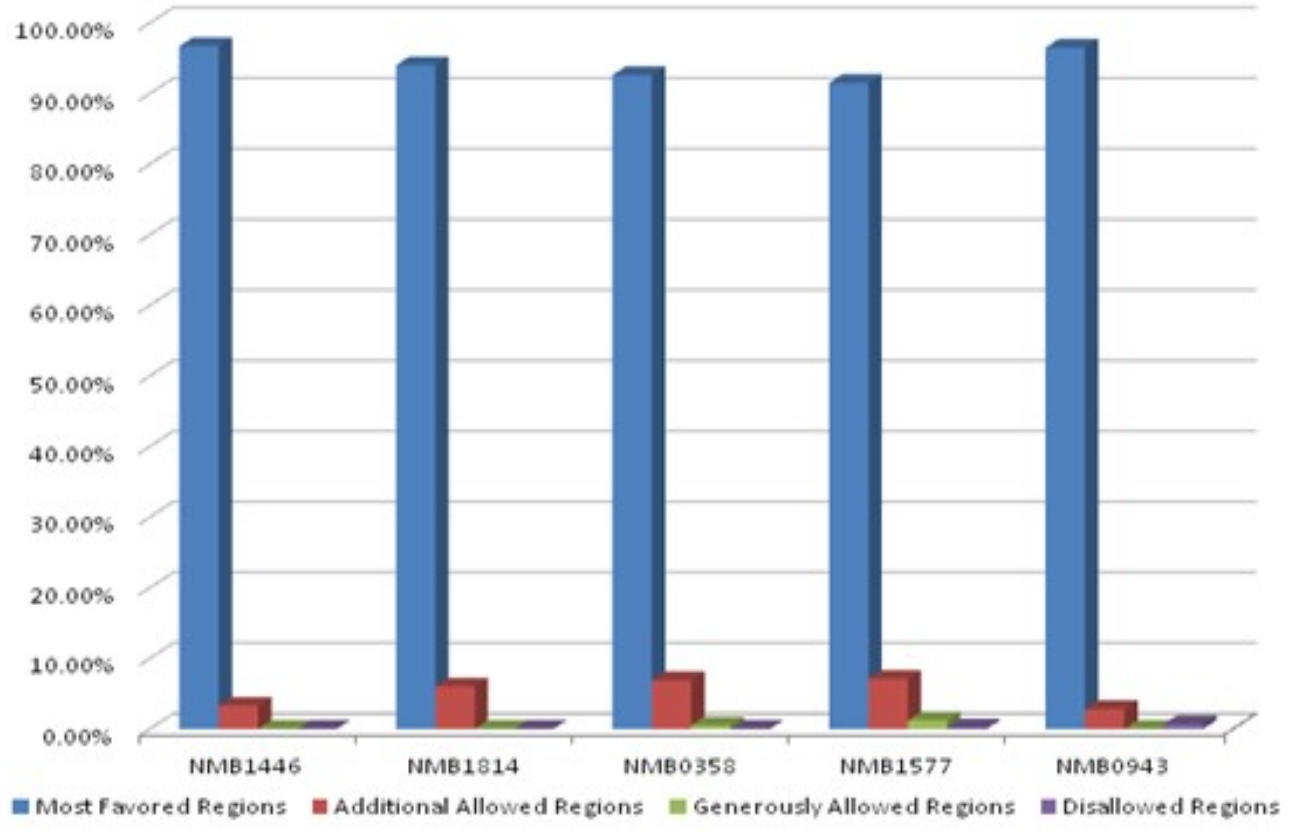

Fig. 2: Stereochemical analysis of the models using Ramachandran plot. Percentage residues in different regions are shown.

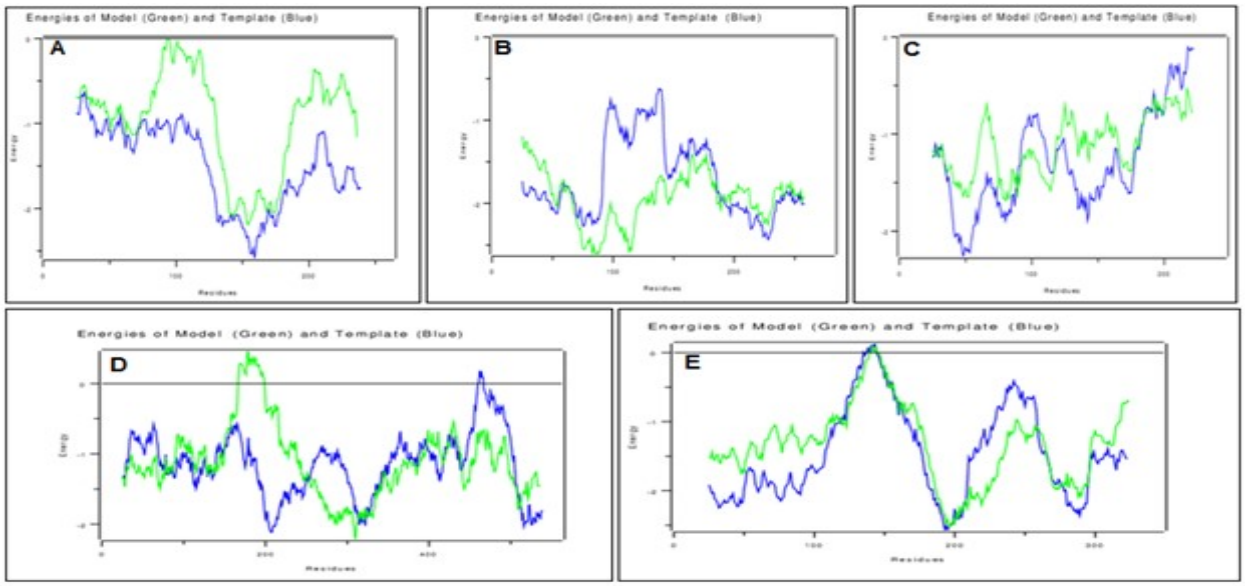

Fig. 3: Energy profiles of all the models with their respective templates using PROSA standalone software.

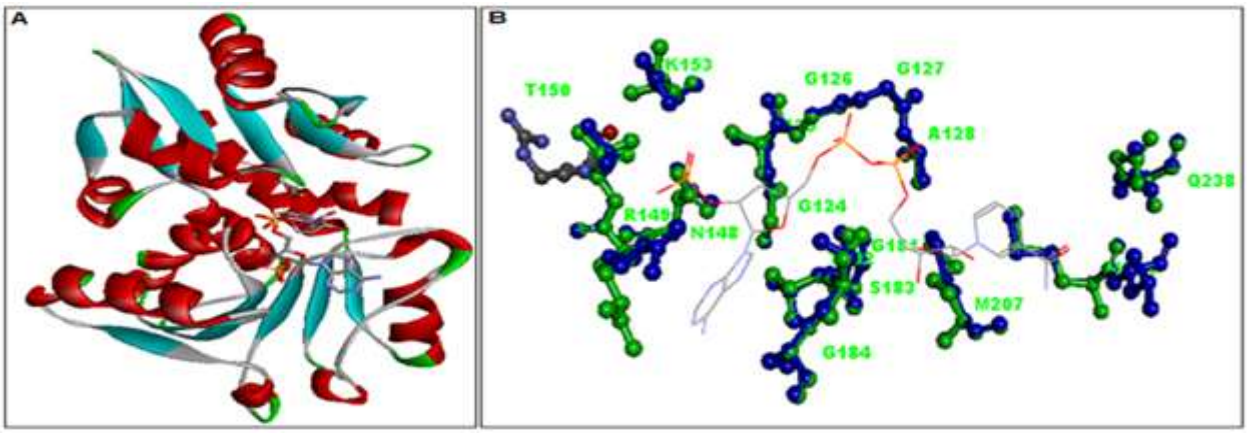

Fig. 4: Homology model of *N. meningitidis* Shikimate dehydrogenase (A); Superposed active site residues of the template [green] and the model [blue] with NADP+ in stick representation(B)

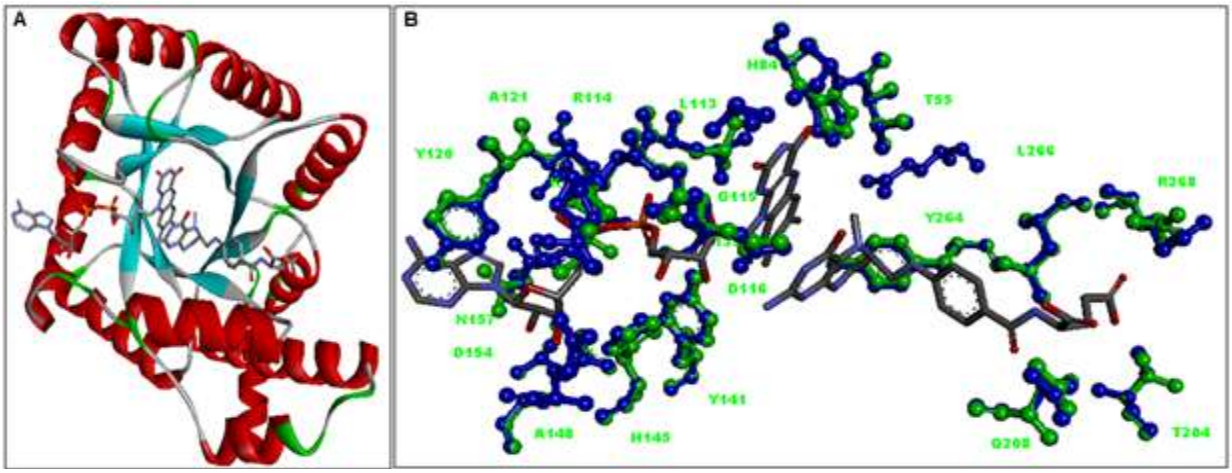

Fig. 5: Homology Model *N. meningitidis* 5, 10-methylenetetrahydrofolate reductase (A); Superposed active site residues of the template [green] and the model [blue] (B); Methyl tetrahydrofolate and FAD+ are shown in stick representation.

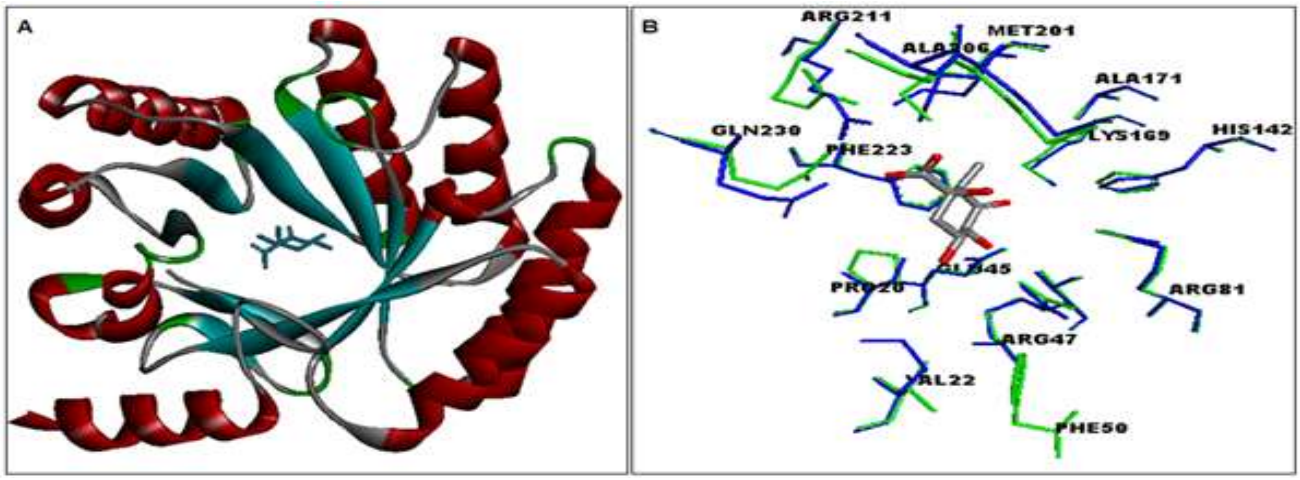

Fig. 6: Homology model of *N. meningitidis* 3-dehydroquinate dehydratase (A); Superposed active site residues of the template [green] and the model [blue] (B); 2-methyl dehydroquinate is shown in stick representation.

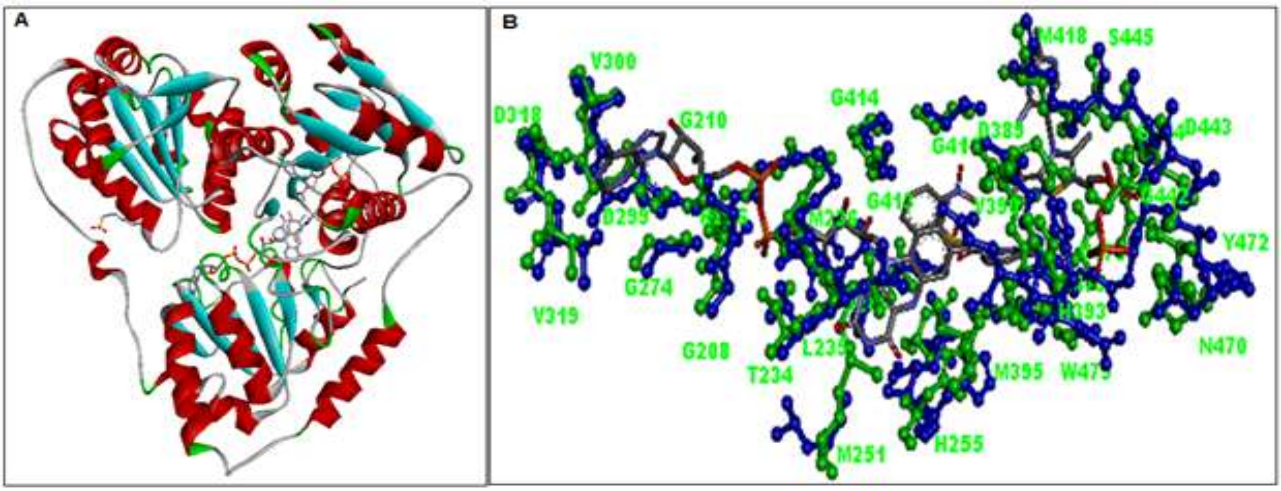

Fig. 7: Homology model *N. meningitidis* acetolactate synthase (A); Superposed active site residues of the template [green] and the model [blue] (B); the inhibitor monosulfuron is shown in stick representation.

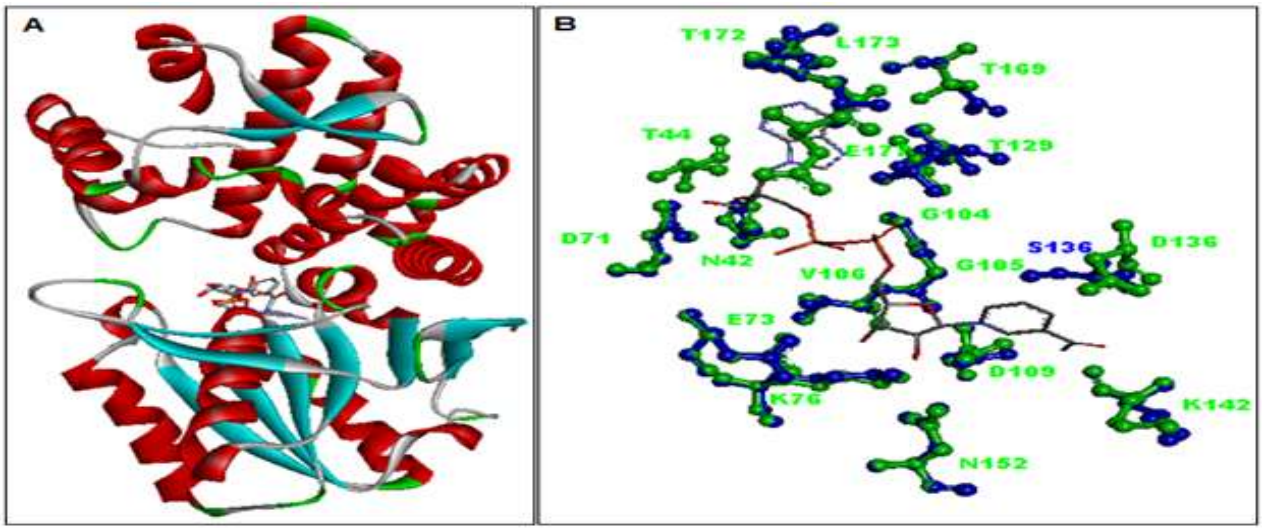

Fig. 8: Homology model *N. meningitidis* 3-dehydroquinate synthase (A); Superposed active site residues of the template [green] and the model [blue] (B); NAD+ is shown in stick representation.

Structural studies of 3-dehydroquinate dehydratase

N. meningitidis 3-dehydroquinate dehydratase (aroD) is involved in the third step of the chorismate pathway, which leads to the biosynthesis of aromatic amino acids. It catalyzes the cis-dehydration of 3-dehydroquinate (DHQ) and introduces the first double bond of the aromatic ring to yield 3-dehydroshikimate. Chain A of *Salmonella Typhi* Type I Dehydroquinase complexed with its Inhibitor, 3-dehydroquinic Acid derivative, having PDB id 4CNO (Maneiro *et al*., 2014) was selected. The structure had 98% coverage and 46% identity. From the PSIPRED, the structure seems predominately composed of Alpha helix with high confidence interval (fig. 1). ProCheck identified almost all regions in the "Most Favored Region" and a small fraction (3.3%) of residues in additional allowed regions (fig. 2). The ProSA calculated energy for all residues, averaged at 50, to be less than zero (fig. 3C). The superposition of two structures gives the RMSD value of 0.335. All these data supported the overall quality and validity of the model (fig. 6A). The active site shows almost identical amino acid residues including some conservative substitutions (fig. 6B).

Structural studies of acetolactate synthase

N. meningitidis acetolactate synthase is responsible to synthesize acetolactate from pyruvate. Chain A of Arabidopsis thaliana acetohydroxyacid synthase in complex with monosulfuron bearing the PDB id 3E9Y (Wang *et al*., 2009) was selected as the template. The query coverage for this template was 97% and sequence identity was 43%. From the PSIPRED, the structure seems predominately composed of Alpha helix with high confidence interval (fig. 1). Model, similar to the template, appeared to consist of three domains. All domains have alpha/beta fold with the ligands located in the center (fig. 7A). Procheck analysis revealed most residues to be concentrated in the allowed region with few in generously allowed region (fig. 2). ProSA calculated few residues to have higher energy while most remain below zero (fig. 3D). Closed analysis revealed that the high energy region showed to be far from active site. The Z score for the model was calculated to be -5.08. Despite some glitches, active sites of the model and template seemed to superimpose well especially with regards to the inhibitor interaction (fig. 7B).

Structural studies of 3-dehydroquinate synthase

3-dehydroquinate synthase catalyzes the conversion of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) to dehydroquinate (DHQ) (Maeda & Dudareva, 2012). Chain A of 3-dehydroquinate Synthase from Acinetobacter Baumannii in complex with NAD+ with PDB id 5EKS (to be published) was selected as a template structure. The template had 98% query coverage and 56% sequence identity with the target sequence. From the PSIPRED, the structure seems predominately composed of Alpha helix with high confidence interval (fig. 1). The constructed model seems to be composed of multiple domains and is predominantly alpha helical structure. Multiple beta strains appeared in the other half of the protein. The ligand NAD was present at the core of the structure. ProSA showed an overall negative energy profile (fig. 3E). ProCheck showed most residues in the most favored region and a small number (6%) in the additionally allowed region (fig. 2). The model showed strict fold conservation compared to the template (fig. 8A). The active site residues seemed to be well conserved except for a D136 -> Ser substitution in *N. meningitidis* 3-dehydroquinate Synthase (fig. 8B).

DISCUSSION

In general, enzymes involved in the biosynthesis of amino acids are essential for the growth and survival of bacteria. The metabolic pathways and transport of amino acids are considered as potential targets for novel antimicrobial agents (Nishida et al, 2016).

The shikimate pathway is essential to plants, bacteria, and fungi and is known to link carbohydrate metabolism with the biosynthesis of aromatic compounds. This seven-step metabolic pathway involves the conversion of phosphoenolpyruvate and erythrose 4-phosphate to chorismate which is the common precursor for the synthesis of folic acid, ubiquinone, vitamins E and K, and aromatic amino acids (Herrmann and Weaver, 1999). Due to the absence of Shikimate pathway in metazoans, the enzymes of this pathway are considered to be important targets for the development of herbicides (Kishore and Shah, 1988) antimicrobials (Davies et al, 1994) and antiparasite (Roberts et al, 1998) agents. The overall fold including three dimensional architecture of active site of *N. meningitidis* shikimate dehydroganease showed high degree resemblance with that of *E.coli* shikimate dehydrogenase suggesting an identical overall catalytic mechanism in these bacteria. Methylenetetrahydrofolate reductase (MTHFR), a flavoprotein, catalyzes the reduction of 5,10-methylenetetrahydrofolate (CH_2-H_4folate) to form methyltetrahydrofolate (CH_3-H_4folate), which acts as the sole methyl donor in the production of methionine amino acid (Jacques et al., 1996). The three dimensional fold of *N. meningitidis* 5, 10-methylenetetrahydrofolate reductases is found to be highly similar with its orthologue in *E.coli*. 100% active site residue conservation between these orthologues suggests same catalytic mechanism. The importance of 3-dehydroquinate dehydratase as an antibacterial drug target is obvious by the fact that certain bacterial strains were turned out to be live oral vaccine just by knocking out this single enzyme (Tacket et al., 1992; Karnell et al., 1993). *N. meningitidis* 3-dehydroquinate dehydratase has got overall identical architecture to that of *S. typhi* including the active site residues. Here, we believe that by inhibiting *N. meningitidis* 3-dehydroquinate dehydratase,

the pathogenicity of this deadly microbe can be prevented.

Attenuated stain of *M. tuberculosis* was generated by knocking out the genes involved in the synthesis of the essential branched-chain amino acids. Acetolactate synthase is the first enzyme in Branch Chain amino acid biosynthesis (Hondalus et al., 2000). Here, we propose that despite of minor differences in the three dimensional fold of *N. meningitidis* acetolactate synthase compared to that of Arabidopsis thaliana, active site residues surrounding the inhibitor were found to be fully conserved suggesting an overall similar catalysis as well as inhibition strategies. 3-Dehydroquinate synthase is the second enzyme in the shikimate pathway, which is an ideal target for new antimicrobials, anti-parasitic agents, and herbicides (Liu et al., 2008). Detailed in-silico analysis of *N. meningitidis* 3-dehydroquinate Synthase model suggested its overall similar tertiary structure and function to that of the *A. Baumannii* enzyme.

CONCLUSION

Homology-based tertiary structure determination and molecular modeling of potential drug targets are valuable strategies which provide imperative contribution for computer-aided drug designing. The overall three dimensional folds as well as the active site architectures of all the target models, i.e. NMB0358, NMB0943, NMB1446, NMB1577 and NMB1814 proteins were quite conserved with respect to their templates indicating similar catalytic mechanisms. Here, we are reporting, for the first time, detailed three dimensional structures of *N. meningitidis* pathogenic factors involved in a crucial cellular metabolic pathway and believe that this structural information of important drug targets would be utilized in computer-aided drug designing in future.

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