Protein Profiling and *In Silico A*nalysis of PPI Network in Meningitis CSF Samples

A Major Project Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of

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Submitted by
Satya Prakash
(DTU/13/MTECH/397)
Delhi Technological University, Delhi, India

Under the Supervision of
Dr. Pravir Kumar

Department of Biotechnology
Delhi Technological University
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road,
Delhi-110042, INDIA

CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"*Protein Profiling and In Silico Analysis of PPI Network in Meningitis CSF Samples*"**, submitted by Satya Prakash (DTU/13/MTECH/397) in partial fulfillment of the requirement for the major project dissertation during M.Tech, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work which is to be carry out by him under my guidance.

Dr. Pravir Kumar
Associate Professor
Department of Bio-Technology
Delhi Technological University

Prof. D. Kumar
Head of Department
Department of Bio-Technology
Delhi Technological University

DECLARATION

I declare that my major project dissertation entitled "*Protein Profiling and In Silico Analysis of PPI Network in Meningitis CSF Samples*" submitted to Department of Biotechnology, Delhi Technological University as a result of the work carried out by me at "Molecular Neuroscience and Functional Genomic Laboratory", Department of Biotechnology.

Place: Delhi

Satya Prakash

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ABBREVIATIONS

Ab – Antibody

ADH – Antidiuretic Hormone

ATP – Adenosine triphosphate

BBB - Blood Brain Barrier

CD - Cell Adhesion

CDC - Centers for Disease Control and Prevention

CSF - Cerebrospinal Fluid

FDA – Food and Drug Administration

Hib - *Haemophilus influenza* type b

HIV - Human Immunodeficiency Virus

HSP – Heat Shock Protein

IgA –Immunoglobulin A

IL – Interleukin

LPS – Lipopolysaccharide

PDB – Protein Data Bank

PPI- Protein Protein Interaction

PRP - Polyribosylribitol Phosphate

sHSP - Small Heat Shock Proteins

SIADH - Syndrome of Inappropriate Antidiuretic Hormone

TNF - Tumor Necrosis Factor

Title - Protein Profiling and *In Silico* Analysis of PPI Network in Meningitis CSF Samples

Name – Satya Prakash

Roll No.-2K13/BME/13

ABSTRACT

Meningitis is a chronic neurodegenerative disorder in which inflammation of meninges occurs that results in leakage of CSF. Inflammation in meninges, which is the protective covering of the brain and spinal cord elicit the meningitis. Meningitis could be life threatening because of the inflammation of the meninges. Some common causes of meningitis include virus, fungi, bacteria, and parasites. In bacterial meningitis, inflammation of the meninges affecting the arachnoid, subarachnoid, and pia matter space that happens in response to bacterial products. Bacterial meningitis is also originated to be contagious. The mode of action of bacterial meningitis is direct contact with the respiratory secretion as well as throat and oral secretion of an infected person, such as coughing, sneezing etc. Most causative agents found to be *Haemophilus influenza, Streptococcus pneumonia*, and *Neisseria meningitides*. Children under the age group of five years get affected by this disease mainly caused by *Haemophilus influenzae* type b. Etiopathology of bacterial meningitis have been altered by vaccination against following bacteria namely *Neisseria meningitides, Streptococcus pneumoniae*, and *Haemophilus influenzae* type b. Vaccines have an essential role in prevention of bacterial meningitis. Vaccines against *Streptococcus pneumonia, Neisseria meningitidis*, and *Haemophilus influenzae* are available at present, but each vaccine is specific for each bacterium and limited to some of the serotypes of each bacterium. For example, vaccines are currently available to inhibit *Haemophilus influenzae* infections due to serotype b but that could not be used for that infection which occurs due to other serotypes. HSPs are conserved proteins found in animals and others, acts as molecular chaperon that gets activated in stress condition and helps in correct folding of proteins. However in case of meningitis, inflammation in meninges ascertains the improper functioning of HSPs. Although in this project I have tried to find out the therapeutic role of two biomolecules i.e., Baicalin and Quercetin which could be used for the cure of meningitis. However, Meropenem is already approved by FDA in 1996 for treating bacterial meningitis. I have taken bexA, ctrA, HPD and SodC that are involved in meningitis and for curing this disease it was tried to retrieve the data required to validate the effectiveness of Quercetin and Baicalin as drug in treatment of meningitis.

INTRODUCTION

INTRODUCTION

During a survey performed in the small population of US in 1960-70s, it was found that pathogens like *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumonia* are the main causing agent of meningitis. However, relatively small populations in these studies and therapeutic strategies were concentrating toward these microorganisms, provided the great chances of isolation of these specific pathogens [1-3]. Three identified pathogens including *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumonia* were contributing for more than 85% of cases of these pathological conditions. Another following laboratory based investigation study done over a population of almost 30 million from five states for all cases of bacterial meningitis in 1986. Investigation was performed for the five common etiological agents of bacterial meningitis and found that bacterial agents such as *Haemophilus influenzae*, *Streptococcus pneumonia* and *Neisseria meningitidis* comprises majority of cases of pathological condition. Bacterial meningitis is moreover a challenging issue in all over the world. Further it has been seen that in countries of African continent, rates of HIV infection is very high and the common cases of meningitis are occurred due to the activity of *Streptococcus pneumonia*, and this has been connected with high mortality rates [4-5]. Bacterial meningitis is predominantly found in young children. The most common pathogens during the neonatal period are Gram negative enteric Bacilli, S*treptococci*, and *Listeria monocytogenes*. In children of age group between 4 months to 15 years, the three major causative organisms namely *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* type b accounts more than 90% of bacterial meningitis. The occurrence and relative frequencies of these bacteria mainly depends on genetic factors and the geographic area. Recently developed and introduction of effectual vaccines against Hib. Several risk factors associated with bacterial meningitis have been identified through Epidemiological studies. During studies it was found that, Boys get affected by bacterial meningitis more frequently than girls. Many studies have reported so far to show the presence of racial differences in the occurrence of bacterial meningitis. Socioeconomic determined factors are responsible for 3-4 times greater risk of bacterial meningitis in Black and Hispanic populations than Caucasians [6-9]. High frequency of bacterial meningitis has been found in native of North America. Every year out of one lakh, 200 get affected by bacterial meningitis in Canada [10].

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Haemophilus influenzae

Results have shown a great decrease in the occurrence of *Haemophilus influenzae* type b meningitis with the use of Hib conjugate vaccines [11-13]. Each vaccine includes polyribosylribitol phosphate (PRP), or portion of the PRP covalently linked with carrier protein. Moreover, the outermost layer of the microorganism is mostly made up of complex polysaccharide; the process of conjugation converts the immunogenic processing of polysaccharide to a T-cell dependent antigen from a T-cell independent and greatly enhances immunogenicity. This vaccine is subjected to all the infants beginning at two months of age with a series of three inoculations, and further followed by booster dose at the age of 13-15 months. Up to 90% cases of Hib meningitis has been reduced during the vaccination in developed countries. In other countries for adequate uptake of vaccine has shown reduction up to 55-80%; this can be observed that conjugate vaccines is capable in minimizing the nasopharyngeal presence of the microorganism and subsequently, can drop the transmission process of microbial infections through herd immunity. A significant decline in *Haemophilus influenzae* type b disease due to the development of herd immunity was evidenced in children at the time period of less than one year of age before the vaccine was allowed for use in this age group [14].

Hib is a primary cause of meningitis in young infants, with maximum death rate around the world [15,16]. In 2007, it was found that only 42% of children had access to Hib vaccines, now almost 90% of the children are consuming the Hib vaccine. A wide analytical study for the use of Hib conjugate vaccines in developing countries is under supervision. Later studies have shown that in other developing countries, the overall vaccine effectiveness has reached up to 95% [17-20].

Streptococcus pneumoniae

Streptococcus pneumoniae is said to be most common cause of bacterial meningitis, which accounts for 58% of total cases in developed countries [21-23]. To lessen the occurrence of pneumococcal meningitis vaccination strategies has been employed. However the suggestion of Hib vaccine for certain high risk groups have shown less prolific results for the prevention of pneumococcal disease, even though 75-85% of the serotypes were restricted to significant valent pneumococcal polysaccharide vaccine isolated from CSF of pneumococcal meningitis patients. Pneumococcal conjugate vaccines were established which included carrier proteins from a nontoxic variant of tetanus toxoid, diphtheria toxin, and an outer membrane protein complex of meningococcal attached to capsular polysaccharides. Later developed heptavalent vaccine includes the seven common

pneumococcal serotypes [24]. With the use of pneumococcal serotypes in the vaccine for reticence of invasive pneumococcal disease, total effectiveness was found to be approximate 90% in fully vaccinated children. Introduction of the heptavalent pneumococcal conjugate vaccine shows a decrease in occurrence rate of the heptavalent pneumococcal conjugate vaccine up to 30% as it was the huge decrease in children less than five years of age (Survey of Nationwide Inpatient Service) [25]. In another study by CDC has proven the lesser chances of pneumococcal meningitis. However, number of meningitis cases caused by non-vaccine included serotypes was increased [26]. Later some of the unapproved studies also show decrease in the occurrence of pneumococcal meningitis where the serotypes have been replaced [27]. Although, various other studies have shown a rise of invasive pneumococcal disease caused due to serotypes which are not included in the heptavalent vaccine, so the study insist to develop a continued surveillance system for the advancement of vaccines with enhanced effectiveness against these other serotypes [28].

Neisseria meningitidis

It is found that approximate 90% of invasive meningococcal disease in the developed countries is sporadic. Occurrence of this disease was up to 30% due to serogroup B and C each and approx. 20% was due to serogroup Y which ultimately caused meningitis in 50% of cases in year 2008 in US [29]. However a contribution of each serogroup in development of meningococcal meningitis was not clearly specified. Many developing countries have shown major outbreaks of meningococcal meningitis initiated largely by serogroup A. Infection rates during these rises can approach less than 1% of the population [30-32]. A high frequency of serogroup X disease was recently notified for Black population, representing up to 50% of 1,140 cases of meningococcal meningitis in 2006 [33]. In the population with a higher risk of invasive meningococcal infection introduction of quadrivalent meningococcal polysaccharide vaccine against serogroups A, C, W135 and Y is supervised to eliminate the infection [34]. The vaccine was not supported for routine use in US since the overall reduced risk of infection, due to failure to protect against serogroup B disease, and the incapability to deliver long-lasting immunity to young children. On the basis of opposing effect of conjugate vaccine over invasive disease caused by Hib and *Streptococcus pneumonia*, conjugate vaccines against specific serogroups of *Neisseria meningitidis* were developed. These conjugated vaccines contain protein such as CRM197, tetanus toxoid, diphtheria toxoid conjugated to meningococcal polysaccharide and induces the development of immunological memory in young children [35]. After the introduction of effective vaccine in an analytical study program in which children and adult subjected with a single dose of the CRM197 meningococcal vaccine, the effectiveness for short term vaccination in children

and adults was up to 90% and 95% correspondingly [36]. In later studies for the first 18 months of the meningococcal C conjugate vaccine program in the United Kingdom, the extensive assumption was found to be 80% over the period 1998-2001 in serogroup C invasive cases, with some inconstancy based on age group [37]. In another case study of adolescents to identify vaccine effectiveness, the protective usefulness of the vaccine was 90% [38]. Presence of serogroup C among subjects age group 15-17 years was also reduced by 60% [39]. These decline in presence persisted for merely two years after vaccine introduction, with no sign of serogroup replacement [40]. In US a quadrivalent meningococcal conjugate vaccine consisting serogroups A, C, W135 and Y conjugated to diphtheria toxoid was approved and was largely proposed for routine immunization [41]. Later routine vaccination was proposed to the age group 10-18 years with single dose [42] and revaccination for the groups at a sustained and advanced risk of meningococcal disease [43]. It is to be believed that protective Ab in adults are likely to persist as long as, and maybe longer than, that after the introduction with meningococcal polysaccharide vaccine [44,45]. Further, the age group of pathogens is also important for their pathogenicity. At certain age they cause or create disease ambiances, and greatly affect the normal population. The major pathogens and their associated age group have been enlisted in following table below.

Pathogens	Age Group
Escherichia coli,, *Listeria monocytogenes* *Streptococcus agalactiae,* *Streptococcus pneumoniae*	0-4 Weeks
Haemophilus influenzae, *Listeria monocytogenes,* *Neisseria meningitides,* *Streptococcus pneumoniae*	1-3 Months
Haemophilus influenzae, *Neisseria meningitidis,* *Streptococcus pneumoniae*	3 Months-18 Years
Neisseria meningitidis, *Streptococcus pneumoniae*	18-50 Years
Listeria monocytogenes, *Streptococcus pneumoniae*	>50 Years

Table No.-1: Pathogens that plays role at different age group

Heat Shock Protein

Most proteins do not fold spontaneously and are prone to aggregation during folding, protein folding efficiency in the cell would be too low to meet biological requirements without intervention [46]. Protein folding in vivo is facilitated by the presence of a large group of related proteins that interact with folding proteins throughout the folding process and beyond. These helper proteins have been termed "molecular chaperones". Molecular chaperones are a diverse group of structurally unrelated proteins that assist in the formation of correct protein structure without becoming a part of the final structure [47]. Protein folding with the aid of molecular chaperones can be referred to as "assisted self-assembly" [48]. Principle that requisite information for the achievement of the native structure is still upheld, as molecular chaperones do not guide folding or impart any information to facilitate folding. Rather, molecular chaperones prevent the association of partially-folded proteins by transiently interacting with regions of the folding intermediate and stabilizing its non-native structure [49]. The folding protein is thereby intercepted before it commits to alternative, unproductive pathways. Molecular chaperones do not act as catalysts in protein folding and their assistance does not increase the rate of folding. Instead, the chaperoning role increases the yield of correctly folded proteins by preventing interactions between molten globule intermediates [50]. Typically, more than 95% of nascent polypeptides fold into their native state under normal cellular conditions just because of the presence of molecular chaperones, or heat shock proteins (HSPs) [51].

The heat shock response was first noted in 1962 with the observation of chromosomal "puffs" corresponding to increased RNA synthesis under elevated temperatures in Drosophila [52]. Some 10 years later, it was shown that these puffs coincided with the expression of a group of proteins which were later termed heat shock proteins [53]. Although functionally related, molecular chaperones are a structurally diverse group of proteins. The five main families of HSPs defined by apparent molecular mass are HSP60, HSP70, HSP90, HSP100 and sHSP (Small Heat Shock Proteins). These HSPs are determined by SDS PAGE. Under physiological conditions, each of these HSP families performs roles that contribute to the functional form of "target" proteins. Some of the HSP groups also perform a range of diverse tasks. Interestingly different stress condition including biological, mechanical, nutritional, physical etc. plays a crucial role in various process like hormonal imbalance, shift from anaerobiosis to aerobiosis, starvation, and inflammation and so on. These processes are pivotal for normal protein biosynthesis, bioenergetics process, and other metabolic activity of body [54]. The table below showing the different stress type and their associated functions or descriptions and elucidate their role in different stress conditions.

Stress Type	Description
Alcohol	Ethanol, methanol, butanol, propanol, octanol
Antibiotics	Puromycin, tetracycline, nalidixic acid, doxorubicion
Biological	Infection, inflammation, fever
Mechanical	Compression, shearing, stretching
Nutritional	Starvation involving multiple or any one of following nutritional components (C,N,$C_6H_{12}O_6$,P and NO_3^-)
Oxygen	Reactive oxygen species, H_2O_2, reperfusion, ischemia
pH	Alkalosis, acidosis, pH shift
Physical	Heat , cold, irradiation, magnetic fields
Psychological	Emotions, emotion related conflicts, hormonal imbalance

Table No.-2: Different nature that induce the stress condition

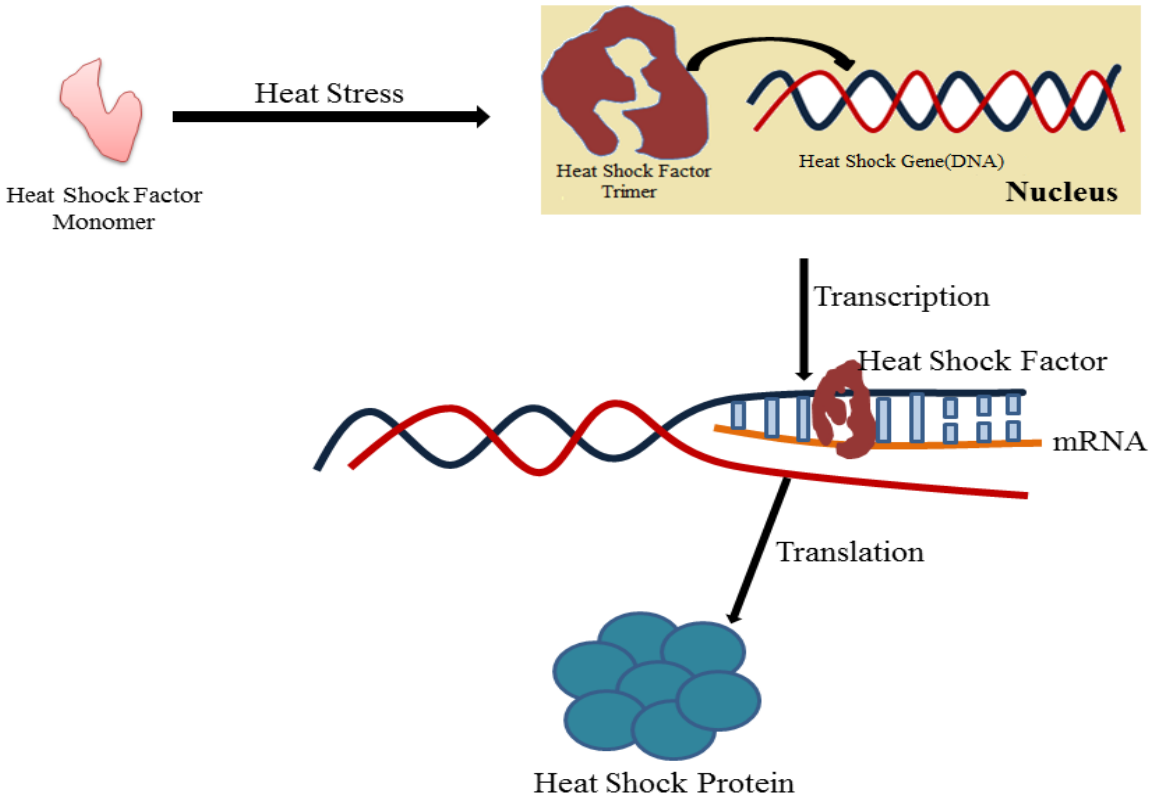


Figure-1: Mechanism showing the activation of heat shock protein

HSP60

The HSP60 (bacterial GroEL) family, also known as the chaperonin family, provides folding assistance for polypeptides that cannot achieve their native structure in the cellular environment, despite stabilisation by the HSP70 chaperones. It is estimated that approximately 15% of all bacterial proteins require interaction with HSP60 to fold correctly [55]. HSP60 is arranged in two heptameric discs with seven-fold rotational symmetry [56,57], forming a large, open cavity that can accommodate folding proteins of up to approximately 75 kDa [58,59]. Molten globule intermediates bind to hydrophobic binding sites on the inner apical surface of one of the HSP60 rings [60-62]. Binding of ATP allows the association of the co-chaperone HSP10 (bacterial GroES), a single heptameric ring of ~10 kDa subunits [63], which displaces the folding protein into the HSP60 cylinder and acts as a lid [75]. Protein folding therefore proceeds in isolation, negating the danger of aggregation due to hydrophobic interactions with other folding intermediates [64]. Co-operative hydrolysis of ATP causes conformational changes in the HSP60 subunits that lead to the interior becoming progressively more hydrophilic, thus encouraging the burial of hydrophobic regions of the target protein [65]. ATP hydrolysis in the opposite ring of GroEL triggers the bound HSP10 to dissociate and the target protein to be released into the bulk solution [66]. The polypeptide may fold to its native state within the cage or complete its folding after release from HSP60 [67]. Many proteins require several binding release cycles to attain their native structure. Upon release, these proteins still expose extensive hydrophobicity and are recaptured by HSP60 [68].

HSP70

The HSP70 and HSP60 molecular chaperones families function as a co-operative team to facilitate the correct folding of cellular proteins. HSP70 (bacterial DnaK) is the first molecular chaperone encountered by the folding protein. This chaperone functions as a monomer and recognizes short, extended, hydrophobic-rich segments of the polypeptide chain, thus protecting unfolded polypeptides from interacting either with other regions on the extended chain or other non-native proteins in the cell [69-71]. The chaperone action of HSP70 is dependent on a co-chaperone of the HSP40 (bacterial DnaJ) family and ATP hydrolysis. HSP40 recognizes and binds to the polypeptide as it emerges from the ribosome, targeting ATP-bound HSP70 to the chain [72]. Because HSP70 is able to bind co-translationally to the translating polypeptide, one nascent chain may be stabilized by many HSP70 molecules [73]. Release of the polypeptide from HSP70 is dependent on cofactors and ATP [74]. Binding and release of HSP70 may undergo many cycles until a sufficient length of the chain has been

translated to allow the nascent chain to fold. The polypeptide may then proceed to fold independently or be passed to the HSP60 chaperone machinery for further stabilization [75,76].

HSP90

The HSP90 family is important for the regulation of a wide range of functional proteins associated with cell signaling and is one of the most abundant molecular chaperones [77,78]. HSP90 functions as a dimer [79,80] and operates in association with various co-chaperones and ATP [81-83]. In eukaryotes, HSP90 has been shown to be important for the activation of steroid receptors, transcription factors, kinases and tumor suppressors [84,85] and can act cooperatively with HSP70 in the late stages of folding of steroid hormone receptors [86]. Once folded, the receptor is inactive and only attains its hormone-binding conformation after interaction with HSP90 [87,88]. Importantly, the locations and the molecular size of these heat shock proteins are an important signal for their normal functions. Due to stress environment, these proteins are constitutively expressed in organelle like cytosol and mitochondria, where it triggers their various activities. The table below enlisted these important HSPs and their associated action in different stress condition.

HSP	Molecular Size (kDa)	Location	Functions
HSP10	10	Mitochondria	Cofactor for HSP60
Low molecular weight HSPs	20-30	Cytosol/Nucleus	Regulation and migration of cellular cytoskeleton, regulate vascular tone and vessel wall remodeling.
HSP56	56	Cytosol	It stabilizes the steroid hormone receptor complex by binding with it
HSP60	60	Mitochondria	It is important for folding and assembly of newly imported proteins
HSP72	72	Cytosol/Nucleus	Highly stress inducible (tolerance)
HSP73	73	Cytosol/Nucleus	Constitutively expressed molecular chaperone

Table-3: Short description of different heat shock proteins

Pathogenesis of Infection

Bacterial meningitis causing microorganisms presents different specific properties to promote adherence, colonization and invasion of the mucous membranes of the nasopharynx. Pilli and nonpolar adhesins are virulence factor which mediate the attachment of microorganism to mucous. Many host defense mechanisms evaded in course of attachment. For attachment and invasion, it is important to inactivate secretory IgA and escape from the ciliary clearance mechanisms of the nasopharyngeal mucosa. Bacteria cleaved the human IgA-1 by secreating protease. IgA-1 is the site of adherence and invasion by bacteria and it is dominant immunoglobulin class in the nasopharyngeal mucosa [89, 90]. Endocytotic process in *Neisseria meningitidis* leads to Invasion across the nasopharyngeal mucosa. Separations in the apical tight junctions of columnar epithelial cells in *Haemophilus influenzae* provide another route of invasion [91, 92]. Once the mucosal barrier is crossed, encapsulation is the most important virulence factor to invade the meninges and to overcome additional host defenses to survive in the bloodstream. The polysaccharide capsule helps in preventing classical complement pathway bactericidal activity by inhibiting neutrophil phagocytosis and thus enhances intravascular bacterial survival and replication.

Diagnosis of Bacterial Meningitis

Nonspecific clinical findings such as abnormal temperature, lethargy, and poor feeding predominate in the neonate and in young infants. Characteristic sign in the age group of 3 months to 10 years are alteration of the mental status, nuchal rigidity, and the signs of Kernig and Brudzinski. Presence of a bulging fontanelle is a relatively a characteristic sign of bacterial meningitis but not present in early stage of the disease. A lumbar puncture also called spinal tap is performed to confirm the disease if the slightest evidence through characteristic sign suggests the presence of bacterial meningitis. However, quick diagnosis and commencement of therapy are the most important prognostic factors for positive outcome. Preliminary assessments are mainly based on the CSF characteristics (glucose, lactate, leukocytes, and protein). Structural and metabolic modification reflected by CSF parameters and Gram-staining, such as permeability amplification of BBB, which illustrate the inflammatory response.

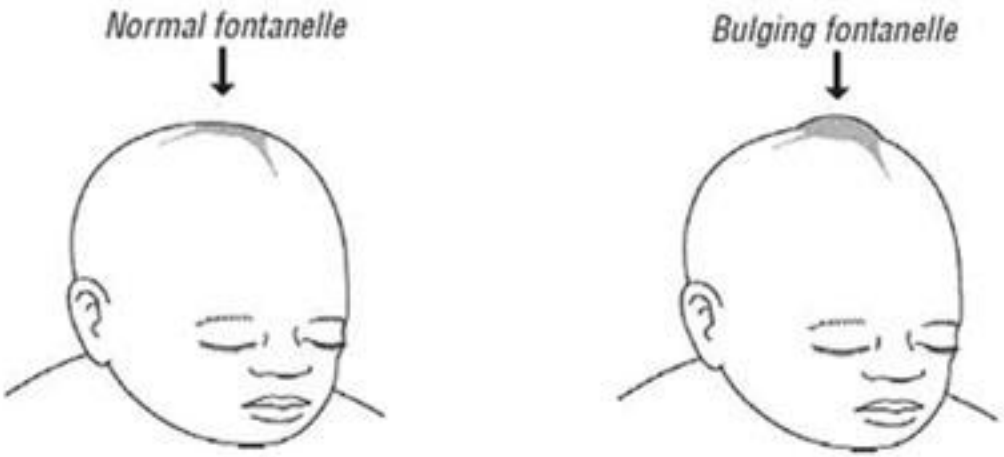


Figure-2: Bulging fontanelle, a characteristic sign of meningitis

Leukocytes

CSF leukocytosis is known to be a distinctive feature of bacterial meningitis. Before reaching the CSF, a crucial action of leukocytes for the adherence of vascular endothelium takes place which is followed by migration across the endothelial monolayer into the CSF compartment. This process mediates through adhesion promoting receptors and the endothelium and ligands located on leukocytes are activated by exposure to lipopolysaccharide (LPS) and cytokines: interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon-γ [93-97]. Replication of Bacterial species or its lysis in the CSF compartment directs endothelial cells to generate IL-8. IL-8 is an effective chemo attracting agent [98]. Three lectin carbohydrate binding molecules called selectins, granule membrane protein-140 (CD62P), endothelial cell adhesion molecule-I (CD62E), and leukocyte adhesion molecule-1 (CD62L) facilitates the initial reversible adherence of leukocytes to endothelial cells. Each selectin molecule identifies precise carbohydrate sequences on either leukocytes or endothelial cells. Leukocytes are first observed to roll along endothelial cells adjacent to the extravascular site of inflammation. Studies indicate that selectins are involved in the leukocyte rolling. Strong adhesion mediates the binding of integrins to receptors that are available on endothelium. The integrin family of adhesion receptors is composed of heterodimeric glycoproteins having α and β subunit. They may be categorized on the basis of their β subunit, e.g. β_2 integrins (CD11a/CD18a and CD11b/CD18) and β_7 integrins (CD49d/β_7) [99]. By cleavage of the selectin from the cell surface or due to inhibition of its binding, the early selectin adhesion decreases with continued cytokine stimulation. At this instant β_2 and β_7 integrin facilitated neutrophils adherence to intercellular adhesion molecules (CD54 and CD102) and vascular cell adhesion molecules (CDI06) on endothelial cells is directed. Successively, leukocytes navigate the cerebral capillary endothelium through diapedesis [100].

Glucose and lactate

The fluctuation in the levels of glucose and lactate are not only influenced by living bacteria and leukocytes but the metabolism of anaerobic brain in bacterial meningitis is also added to the growth of elevated levels of CSF lactate concentration and hypoglycorrhachia [101]. It appears that local changes in the brain due to ischaemia or mediated by humoral factors, causes an increase in the lactate production. Experimental canine meningitis validated the additional explanation for the development of hypoglycorrhachia. In these animals, the inhibition of carrier mediated transport across the BBB is indicated by low level of CSF glucose. [102, 103]. The elevated usage of glucose in the brain and the abnormal glucose transport across the BBB may contribute to hypoglycorrhachia [104].

Protein

The elevated concentration of protein in CSF during bacterial meningitis is caused by an increased permeability of the BBB. A uniform response consisting of an early and sustained increase in formation of pinocytotic vesicles and a progressive increase in the separation of tight junctions between endothelial cells is observed during the course of experimental meningitis [105]. The cytokine endothelium leukocyte interaction is probably responsible for the disruption of the barrier by opening intercellular junctions and thereby permitting the passage of serum proteins into the subarachnoidal space.

Therapy

Antimicrobial therapy

For many years, combination of chloramphenicol and ampicillin has been known as an effective practical therapy of bacterial meningitis. In many developed countries use of ampicillin has been limited due to the production of anti-ampicillin substance β-lactamase by *Haemophilus influenzae*. For all participating countries a collaborative data for such European study have shown a mean rate of resistance of 15% [106]. In preceding analysis, sporadic cases of chloramphenicol resistant *Haemophilus influenzae* have been recognized and collective resistances to β-lactams and chloramphenicol have been increased [107-112]. Resistance of *Neisseria meningitides* to β-lactam antibiotics is also found progressively. Penicillin resistant *Neisseria meningitidis* is commonly found in Spain [113]. Infections caused by resistant pathogens are comparatively more dangerous than susceptible pathogens because resistant pathogen relatively results in higher rates of mortality. About the selection of primary empiric therapy of childhood meningitis is still missing due to lack of consent among experts. In children, 3rd generation cephalosporins have become vital antibiotics to cure

bacterial meningitis [114-117]. Ceftazidime, cefotaxime and ceftriaxone are most studied cephalosporins and these are highly potent against *Haemophilus influenza*, *Neisseria meningitidis* and *Streptococcus pneumonia* [118-120]. However, *Listeria monocytogenes, Streptococcus faecalis* and methicillin resistant staphylococci are resistant to cephalosporins. Ampicillin against *Listeria monocytogenes* and enterococci should be used to reduce the influence of bacterial meningitis in children up to the age group of 3 months. Identification and determination of the etiological agent and its susceptibility are the key factor to decide drug and its dose for the treatment of meningitis. Clinical response of the patients decides the duration of therapy, generally for *Haemophilus influenzae* and pneumococcal meningitis, 10 days are considered while 7 days for meningococcal infections [121].

Fluid restriction

Adequate amount of CSF required to prevent SIADH lacks in children with bacterial meningitis [122,123]. However, in case of infants with bacterial meningitis shows the higher levels of ADH, which may also be described as a suitable response to intravascular volume reduction rather than dysfunction of hypothalamic pituitary axis [124]. Secretion of ADH to sustain sufficient cerebral blood flow compensates the loss of cerebrovascular autoregulation in bacterial meningitis. Cerebral perfusion pressures determined by the difference between the intracranial pressures and mean arterial blood pressure, which plays important role in determining the cerebral blood flow during bacterial meningitis. An increased in anaerobic glycolysis of the brain, decrease in mean arterial blood pressure, and significantly decrease in cerebral blood flow have been found due to fluid restriction. However, brain edema is not influenced by fluid regimen [101,125]. Reduction in cerebral blood flow deteriorates the neurological outcome. Meningitic patient who fulfill the diagnostic criteria for SIADH have limited fluid restriction.

Vaccination

Vaccines against *Haemophilus influenzae* type b are available in the market. These vaccines are effective and immunogenic in treatment of meningitis throughout span of the highest occurrence of meningitis caused by Hib, with low side effects [126-133]. Therefore, many countries have introduced childhood vaccination programs for Hib vaccination, resulting 90% reduction in *Haemophilus influenzae* meningitis [128,134]. In North, Latin America and European countries main cause of Meningococcal disease is *Neisseria meningitidis*; serogroup B along with other serogroups of *Streptococcus pneumonia* which affects a significant number of children. In adults A, C, W135, and Y polysaccharide non-conjugate tetravalent meningococcal vaccine has found to

be immunogenic and safe, but shows opposite response in young infants which limits its use. Subsequently, the use of serogroup B polysaccharide vaccine is limited in humans as it is poorly immunogenic [135,136]. Later field trials also have confirmed only the partial protection against group B infection with the use of such vaccines [137,138]. Different antigenic proteins coupled with significant serotype vaccines for pneumococcal diseases are now open [139].

METHODOLOGY

METHODOLOGY

Retrieval and Visualization of 3D structure

To visualize the 3D structure of the meningitis disease related proteins bexA, ctrA, HPD and SodC. Templates with PDB ID: 4K6I, 1CGI, 4J5R and 4OH2 were identified for meningitic related proteins bexA, ctrA, HPD and SodC respectively using Protein Data Bank (PDB). Once the PDB ID of proteins was identified, these were visualized using pymol.

RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was used for visualizing the Ramachandran Plot of proteins for the structural evaluation and stereochemical analysis of proteins.

Protein-Protein Interaction Network of HPD Proteins

To visualize the protein-protein interaction of HPD protein, an online tool String database is used. Name of protein was uploaded in the search tool of this database and selecting particular organism as per required, it display the interaction of various proteins with that particular searched protein.

Lipinski Filter Analysis of Screened Drugs (Biomolecules)

Another online tool Sanjeevini (http://www.scfbio-iitd.res.in/sanjeevini/sanjeevini.jsp) is used to get the information of drugs with the help of Lipinski Rule.

Lipinski rule (or Lipinski rule of five) helps to differentiate drug and nondrug like molecules. It is used to identify the possibility of success or failure due to drug likeness for molecules fulfilling with two or more of the following rules

a) Molecular mass should be <500 Dalton.

b) High lipophilicity (expressed as logP less than 5).

c) Less than 5 hydrogen bond donors.

d) Less than 10 hydrogen bond acceptors.

e) Molar refractivity should be between 40 -130.

Active Site Prediction

Dogsite Scorer (www.dogsite.zbh.uni-hamburg.de/) was used to predict the active sites of protein. This server calculates the possible headerpockets. PDB file of proteins was uploaded in the server and it showed the headerpockets with their binding site atoms present in proteins and involved in binding with ligands.

Ligand Optimization and Docking Study

Sdf files of ligands along with their physical and chemical properties were retrieved from PubChem Compound Database (http://www.ncbi.nlm.nih.gov/pccompound). These sdf files converted into pdb format with the help OpenBabel tool. These ligand files and protein pdb files were imported to Hex 8.0 tool for docking. Hex tool was used to calculate the E. values of all the retrieved proteins bind to drug. Molecules with the lowest E. values have higher affinity towards particular protein.

RESULTS

RESULTS

3 D Structure of Proteins

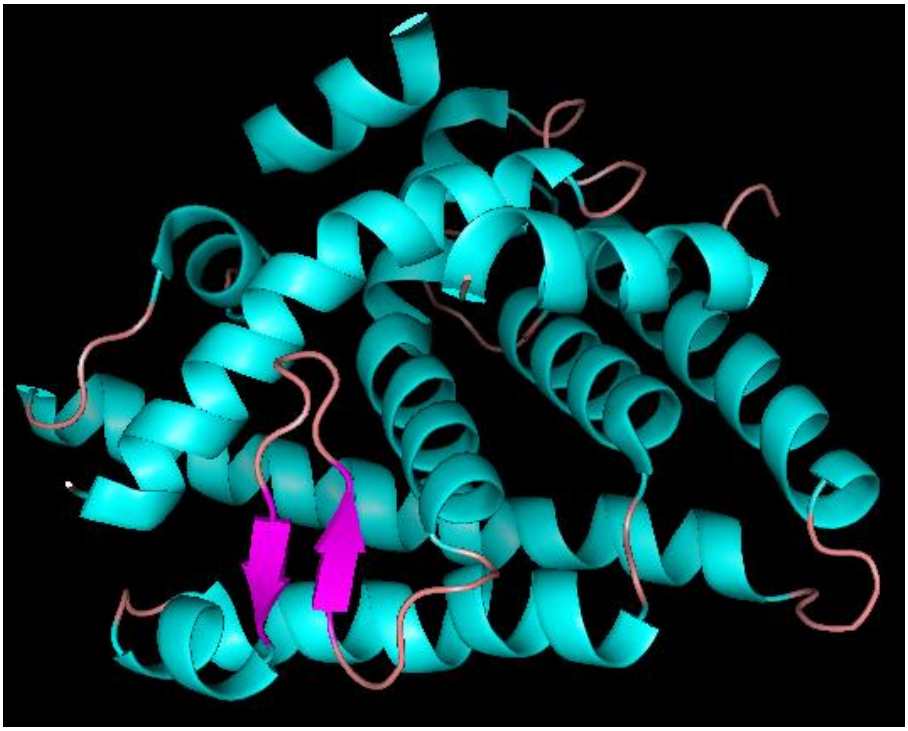


Figure-3: BexA pdb id: 4K6I

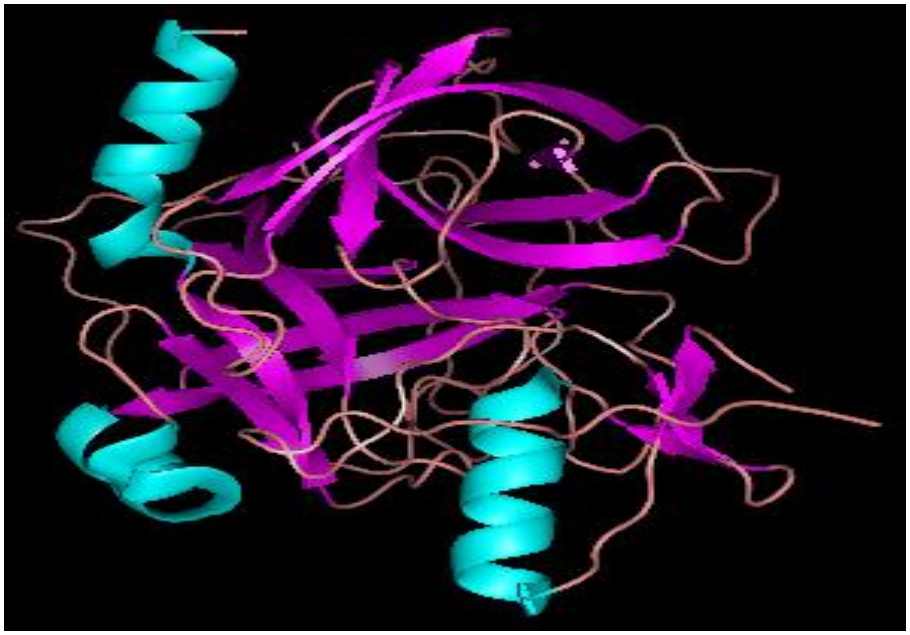


Figure-4: CtrA pdb id: 1CGI

Figure-5: HPD pdb id: 4J5R

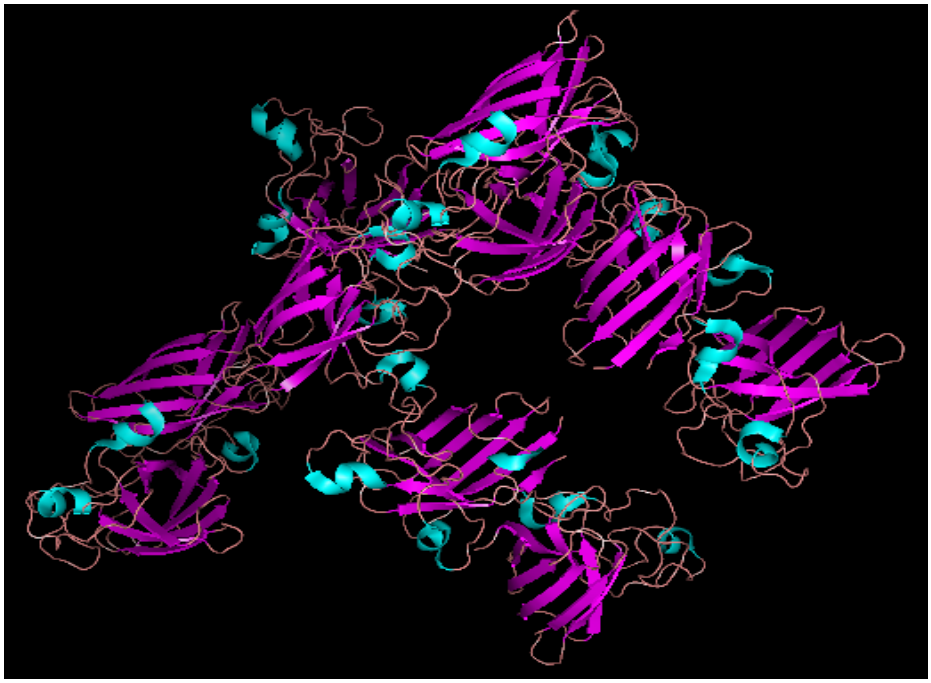


Figure-6: SODC pdb id: 4OH2

3D structure of BexA, CtrA, HPD and SODC were generated using pymol in which alpha helix is shown in skyblue colour, beta sheets in magenta colour and loops in dirtyviolet colour.

Ramachandran Plot of Proteins

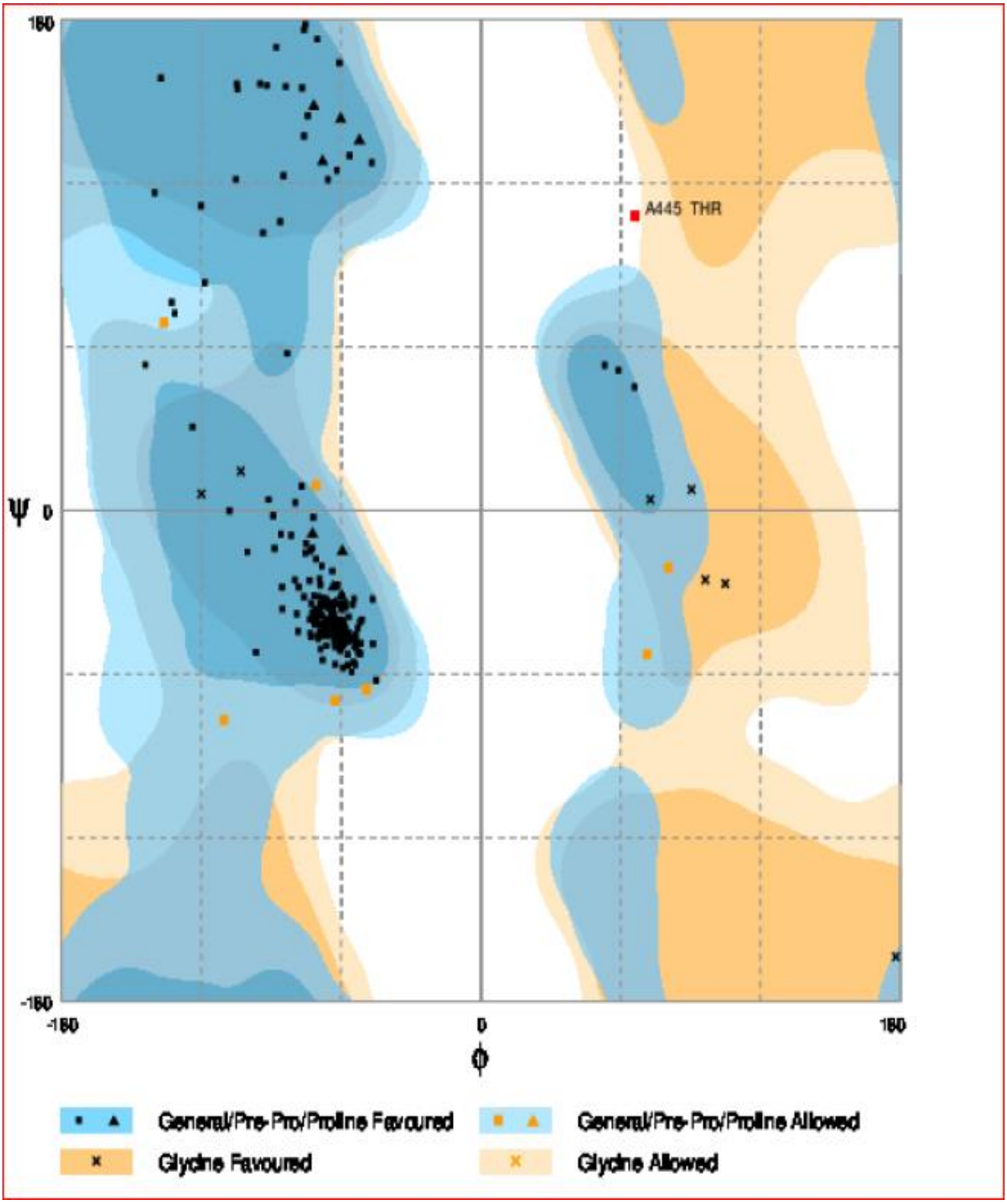


Figure-7: Ramachandra Plot of BexA pdb id: 4K6I

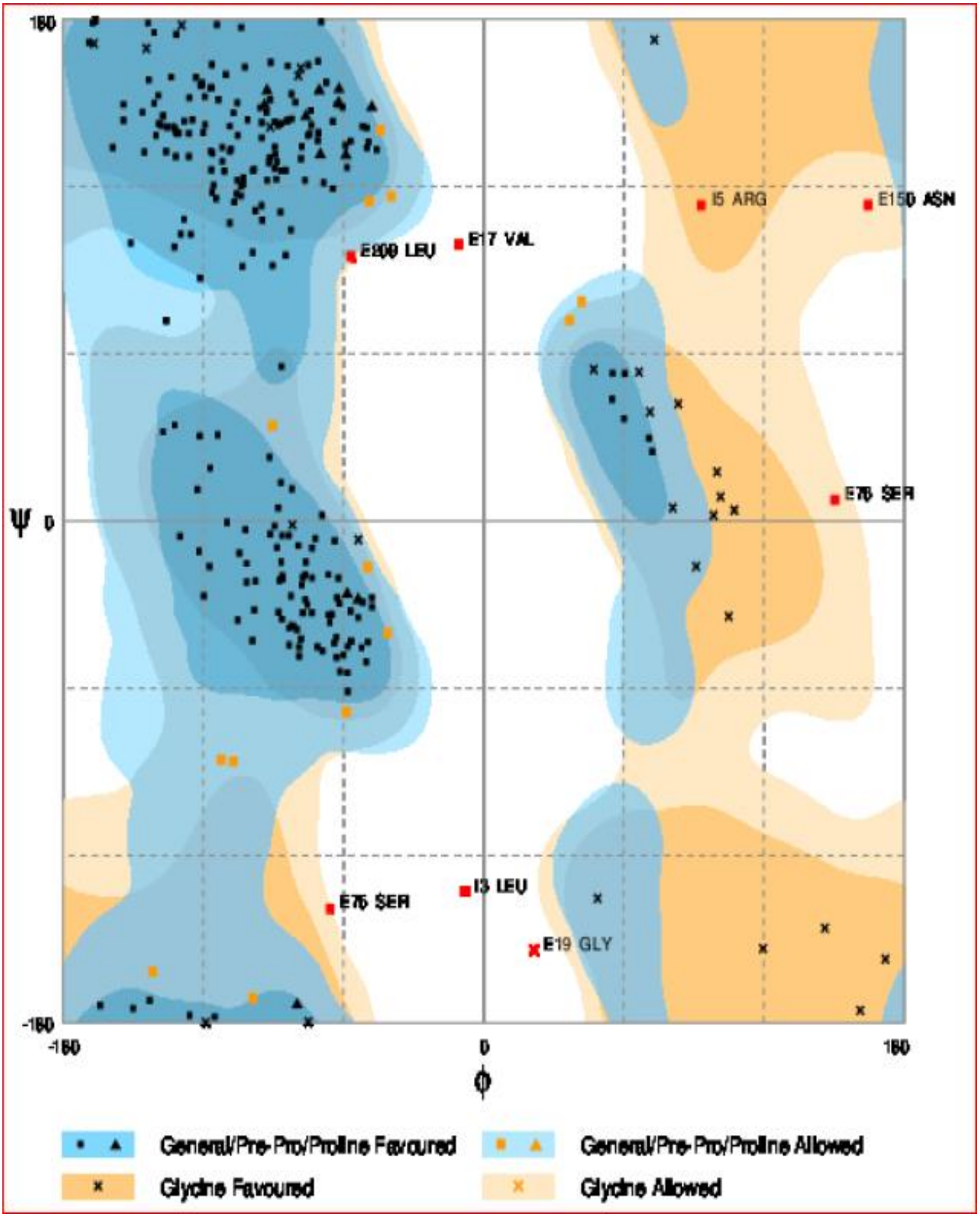


Figure-8: Ramachandra Plot of CtrA pdb id: 1CGI

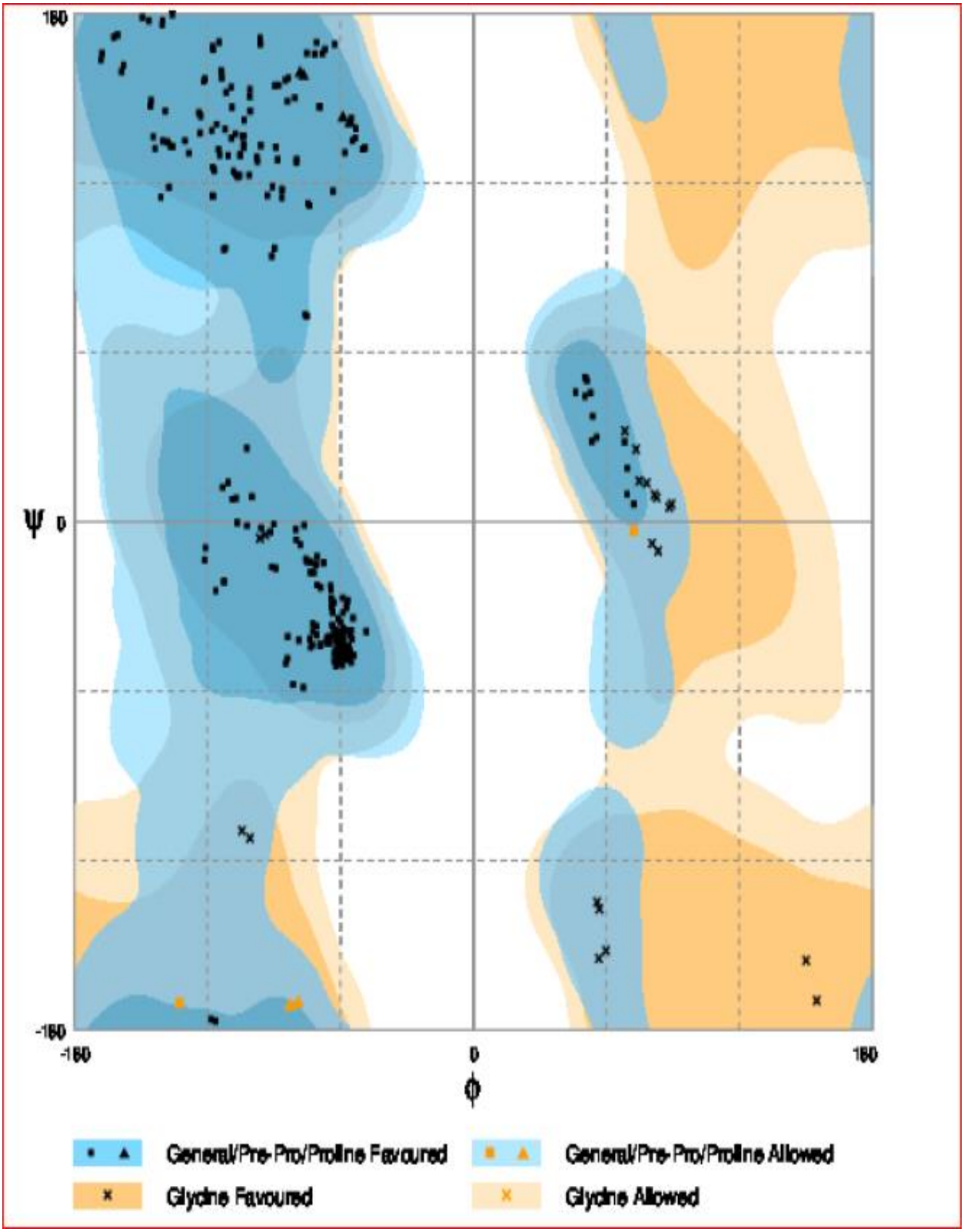


Figure-9: Ramachandra Plot of HPD pdb id: 4J5R

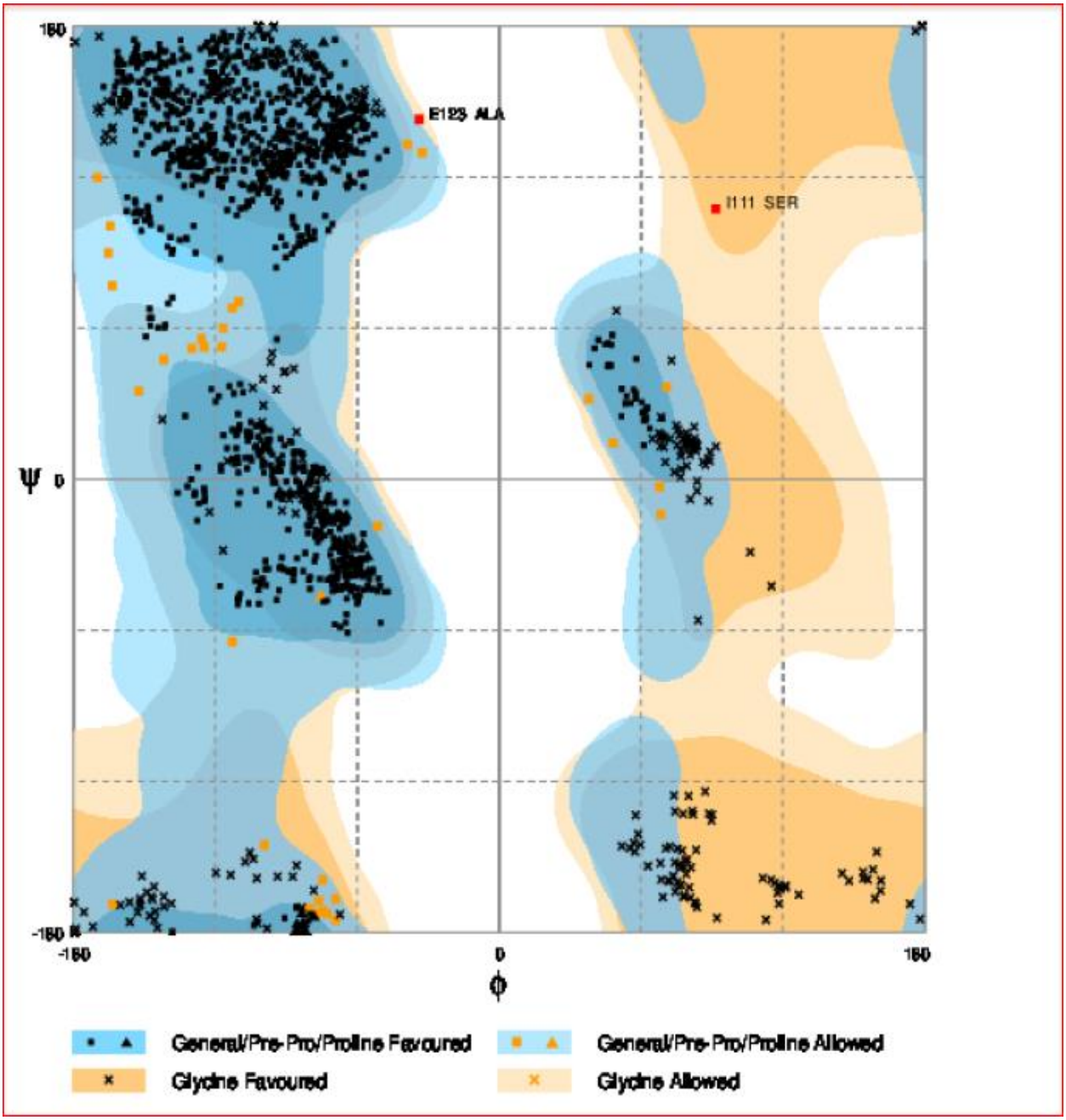


Figure-10: Ramachandra Plot of SodC pdb id: 4OH2

Ramachandran plots were obtained for BexA, CtrA, HPD and SodC for quality assessment. RAMPAGE displayed 96.8% of residues in the most favored regions, 3.1% residues in additionally allowed and 0.1% disallowed regions, for BexA, 95.9% of residues in the most favored regions, 3.5% residues in additionally allowed and 0.6% disallowed regions, for CtrA, 98.8% of residues in the most favored regions, 0.8% residues in additionally allowed and 0.4% disallowed regions for HPD and in case of SODC 96.7% of residues in the most favored regions, 3.3% residues in additionally allowed and no residues in disallowed regions.

PPI Interaction of HPD Protein

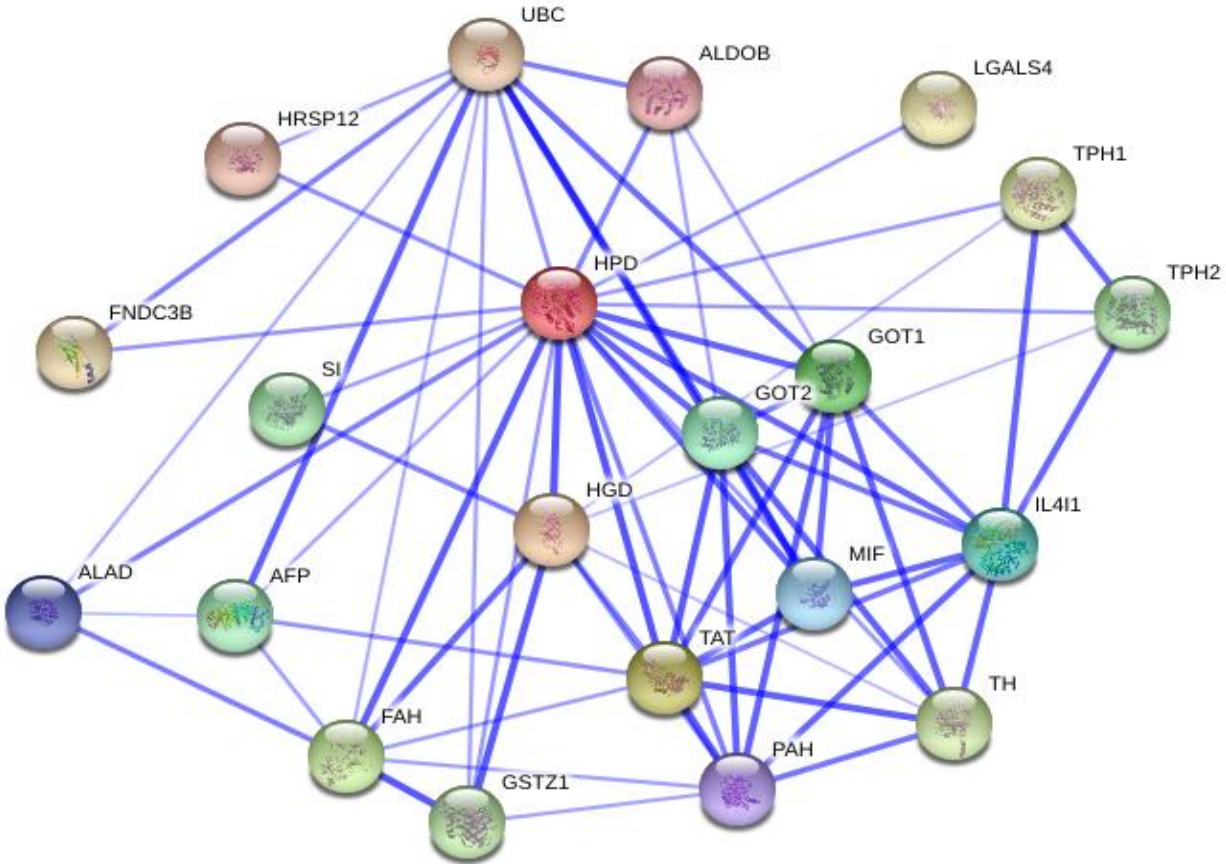


Figure-11: Interaction of HPD with other Proteins

Protein–Protein interaction (PPI) network of human HPD protein was obtain. This interaction displayed that HPD had a functional interaction with HRSP12, UBC, TAT, GOT1, GOT2, MIF, IL4I1, HGD and FAH etc. HPD play an important role in degradation of tyrosine. UBC (Ubiquitin C) and HRSP12 (Heat Responsive Protein 12) are the important chaperons that may be involve in upregulate or downregulate of HPD.

3D Structure of Biomolecules

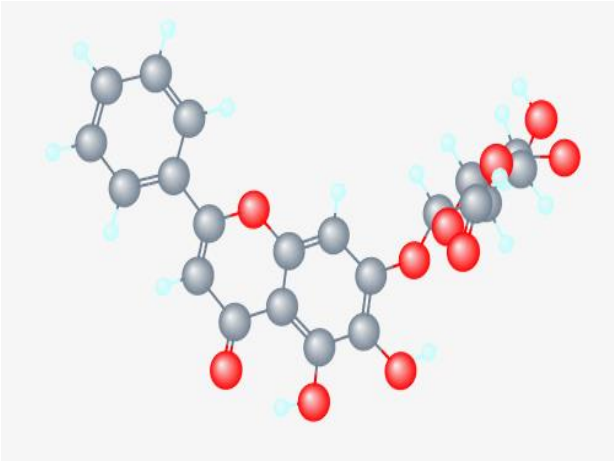


Figure-12: Baicalin

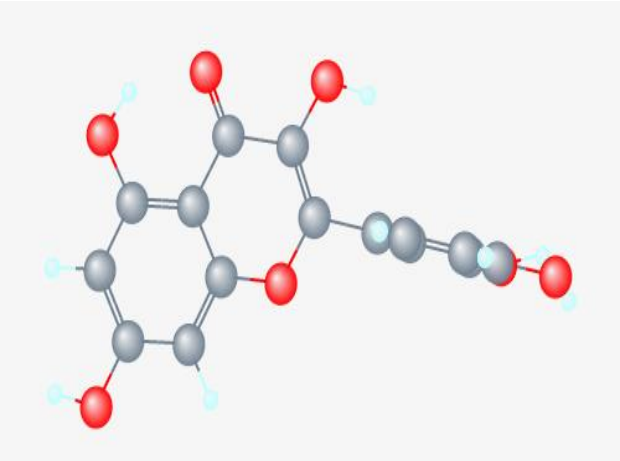


Figure-13: Quercetin

Physical and Chemical Properties of Biomolecules

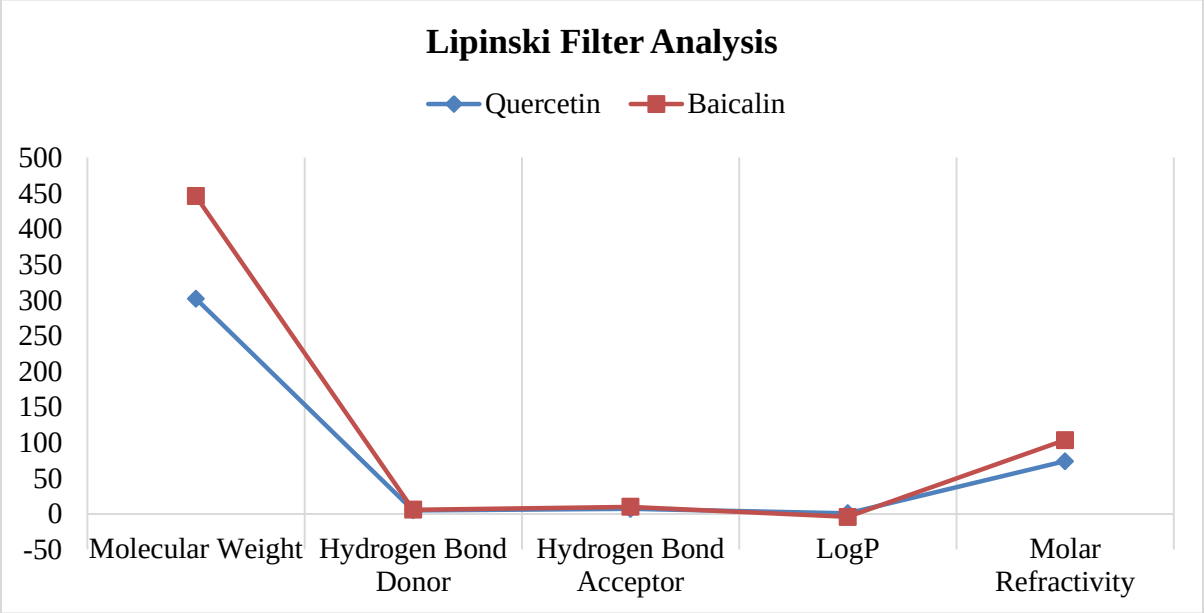
Properties	Baicalin	Quercetin
Molecular Weight	446.36102 g/mol	302.2357 g/mol
Molecular Formula	$C_{21}H_{18}O_{11}$	$C_{15}H_{10}O_7$
XLogP3	1.1	1.5
Hydrogen Bond Donor Count	6	5
Hydrogen Bond Acceptor Count	11	7
Rotatable Bond Count	4	1
Exact Mass	446.084911 g/mol	302.042653 g/mol
Monoisotopic Mass	446.084911 g/mol	302.042653 g/mol
Topological Polar Surface Area	183 A^2	127 A^2
Heavy Atom Count	32	22
Complexity	748	488
Defined Atom Stereocenter Count	5	0
Covalently-Bonded Unit Count	1	1

Table-4: Properties of Baicalin and Quercetin

Lipinski filter analysis of screened drugs

Biomolecules	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	LogP	Molar Refractivity
Baicalin	446	6	10	-4.136	103.652
Quercetin	302	5	7	0.974	73.91

Table-5: Differentiation of biomolecules on the basis of Lipinski Rule



Graph-1: Comparison between different drugs on the basis of Lipinski Rule

Lipinski filter analysis was obtained which revealed that the two biomolecules act as drug on the basis of Lipinski rule of five. When it was analyzed that the two molecules had drug like property, these were then used for docking purposes to understand the interaction of Proteins and the analyzed drug molecule.

Active site prediction

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	1097.98	1138.43	872.08	26.32	0.82
P1	293.63	548.64	377.66	10.51	0.49
P2	287.04	532.48	323.83	12.64	0.56
P3	255.87	360.39	316.77	19.81	0.75

Table-6: Calculation of headerpockets for BexA

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	587.26	701.73	418.75	22.94	0.85
P1	390.78	434.30	257.30	13.45	0.65
P2	224.64	327.47	176.34	13.53	0.55
P3	187.58	312.17	115.32	10.89	0.35
P4	166.53	292.84	119.38	11.24	0.40
P5	140.42	267.77	166.44	7.45	0.22
P6	134.21	279.19	124.35	7.90	0.16
P7	130.24	195.24	106.71	7.59	0.21
P8	114.75	325.08	159.83	7.95	0.22
P9	103.68	164.77	83.58	6.44	0.18

Table-7: Calculation of headerpockets for CtrA

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	748.80	927.90	736.28	20.21	0.83
P1	522.11	655.04	469.97	12.74	0.67
P2	364.29	496.28	307.98	14.25	0.65
P3	327.62	524.06	346.37	14.18	0.58
P4	165.89	323.04	185.30	11.11	0.37
P5	151.49	335.58	240.74	7.59	0.22
P6	144.51	306.61	193.48	10.48	0.33
P7	138.50	240.51	61.56	7.86	0.18
P8	129.92	298.96	235.41	8.59	0.24
P9	119.68	224.65	111.33	7.12	0.15

Table-8: Calculation of headerpockets for HPD

Name	Volume [Å³]	Surface [Å²]	Lipo surface [Å²]	Depth [Å]	Drug Score
P0	1171.36	1316.71	859.55	24.46	0.81
P1	808.27	1138.28	703.19	15.58	0.74
P2	790.18	989.84	552.95	19.04	0.81
P3	755.75	952.98	490.88	19.36	0.81
P4	746.36	868.16	514.42	19.65	0.82
P5	695.93	657.61	435.32	19.83	0.81
P6	658.72	658.11	465.42	19.72	0.81
P7	634.02	919.09	576.43	21.97	0.83
P8	607.94	819.72	481.27	16.33	0.76
P9	576.99	880.58	543.04	15.82	0.74
P10	249.37	498.11	277.04	8.50	0.31
P11	245.89	222.89	115.82	11.70	0.47
P12	236.15	319.00	194.34	15.03	0.58
P13	233.72	381.37	257.34	14.08	0.53
P14	220.85	327.60	216.66	13.10	0.45
P15	216.67	387.69	300.04	12.76	0.50
P16	205.20	242.05	140.06	9.06	0.35
P17	198.94	181.19	102.30	7.34	0.26
P18	187.81	190.16	102.92	9.28	0.35
P19	186.76	275.55	157.67	14.03	0.50
P20	186.76	266.25	175.00	11.87	0.40

Table-9: Calculation of headerpockets for SODC

Dogsite-scorer server was used to detect the ligand binding sites in the form of pockets. This server determines the possible active sites from the 3D structure of the proteins. Active site prediction is useful to determine potential ligand binding sites for molecular docking. Site Volume, Surface Area, Liposurface Area, Depth and Drug Score for all the active sites for BexA, CtrA, HPD and SodC proteins were predicted. Pocket with maximum Drug Score is the most suitable site for the binding of biomolecules.

Docking Result

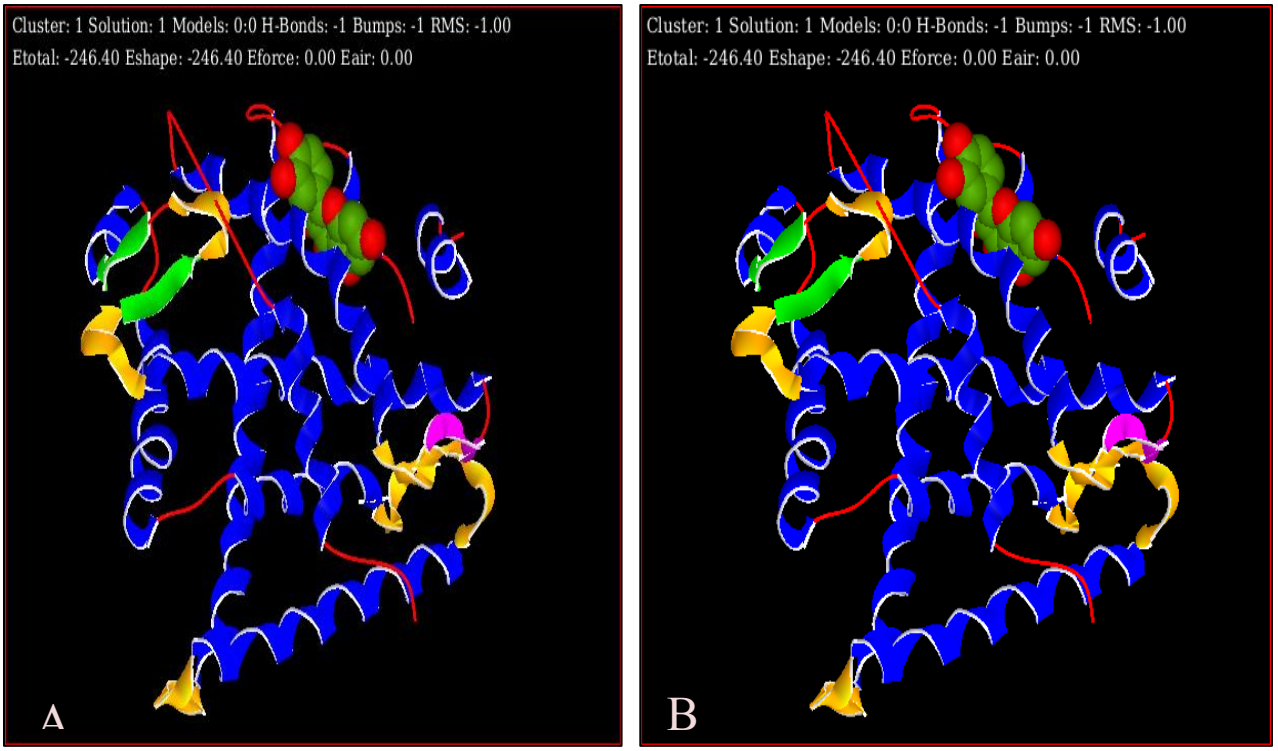


Figure-14: Docking Result of BexA with (A) Baicalin and (B) Quercetin

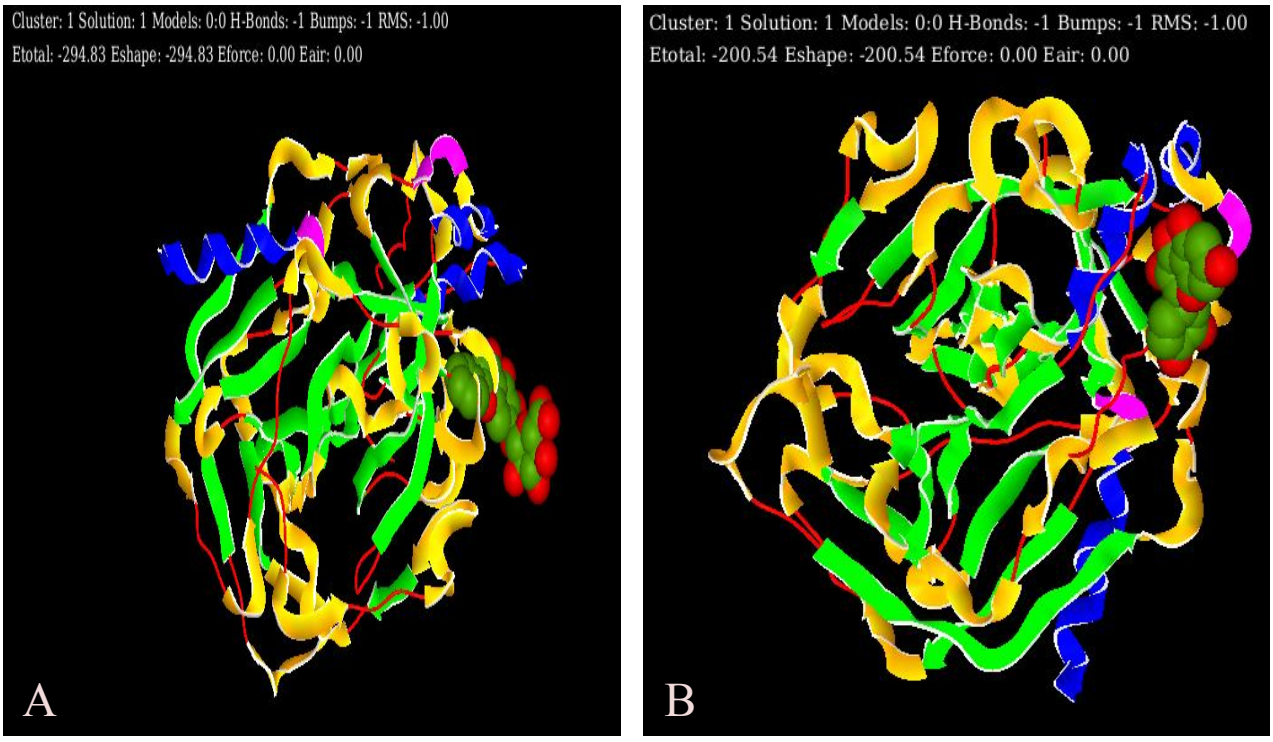


Figure-15: Docking Result of CtrA with (A) Baicalin and (B) Quercetin

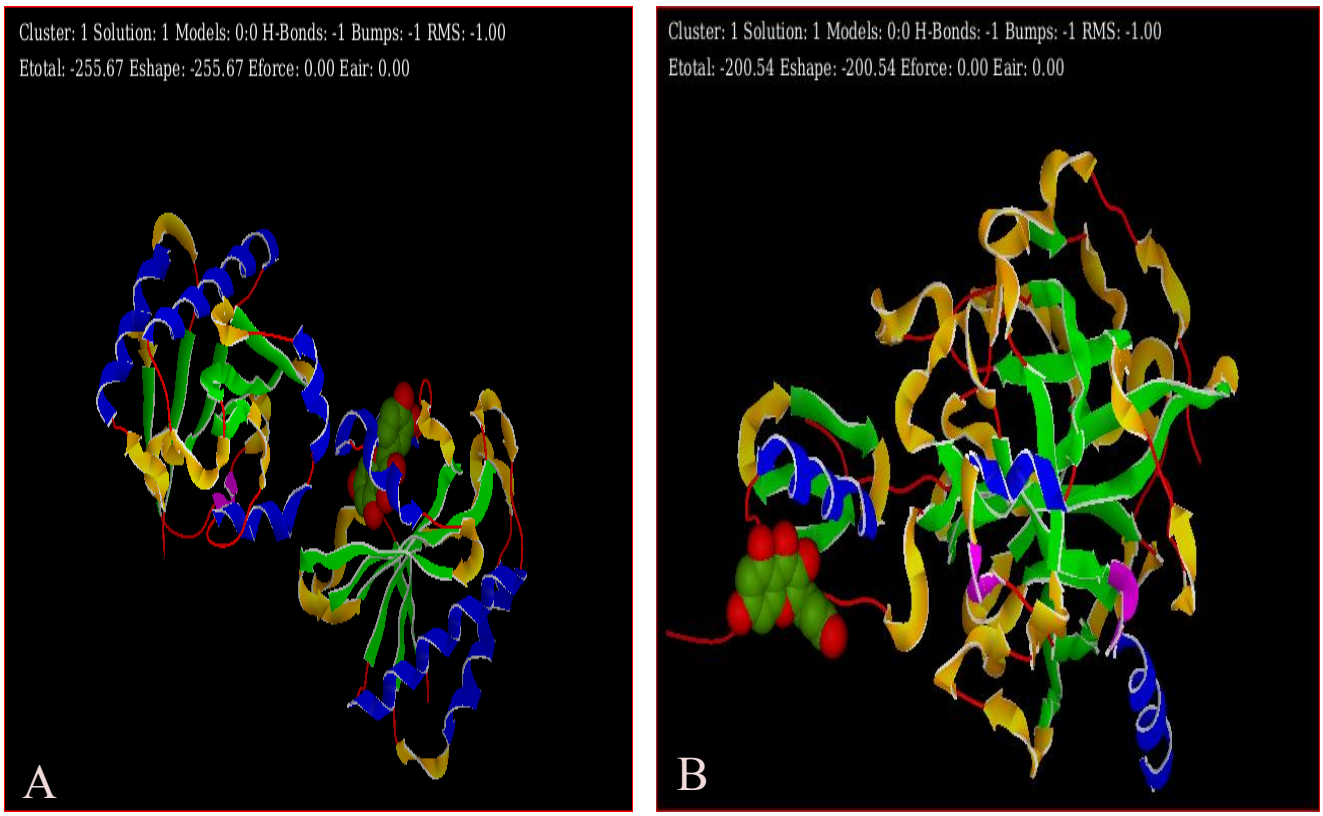


Figure-16: Docking Result of HPD with (A) Baicalin and (B) Quercetin

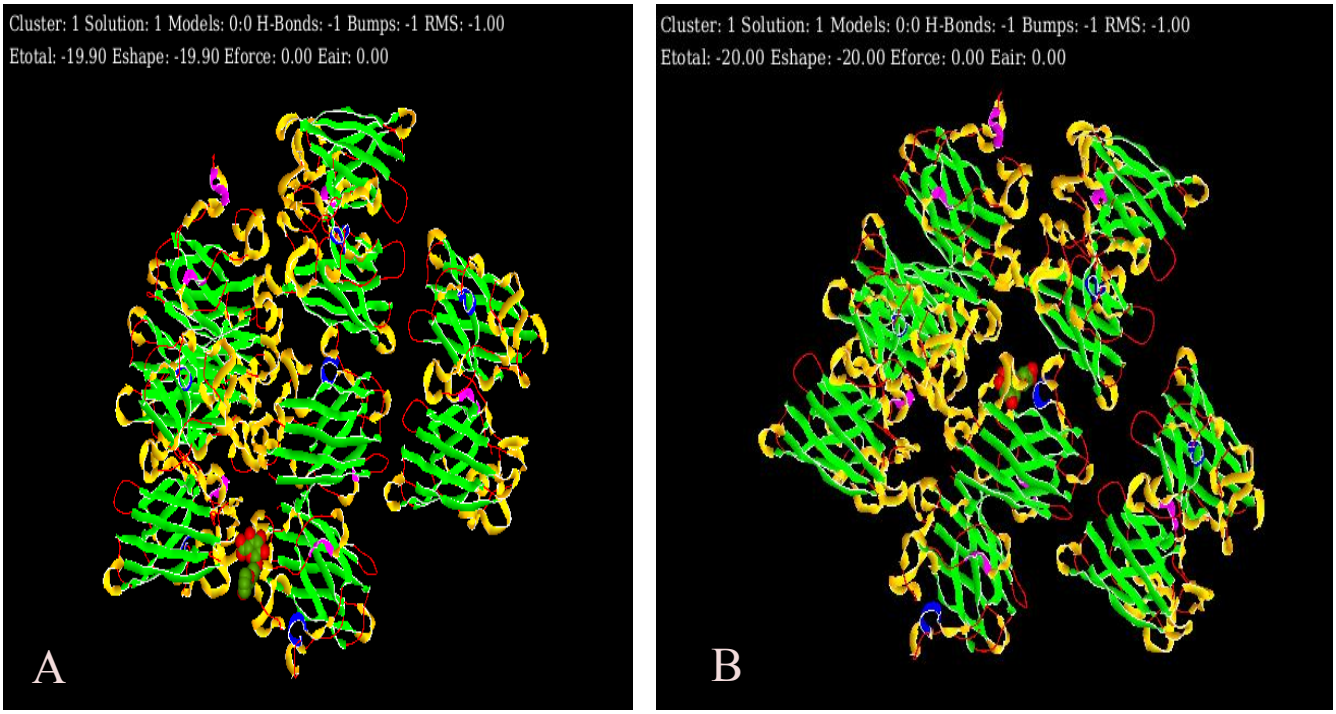
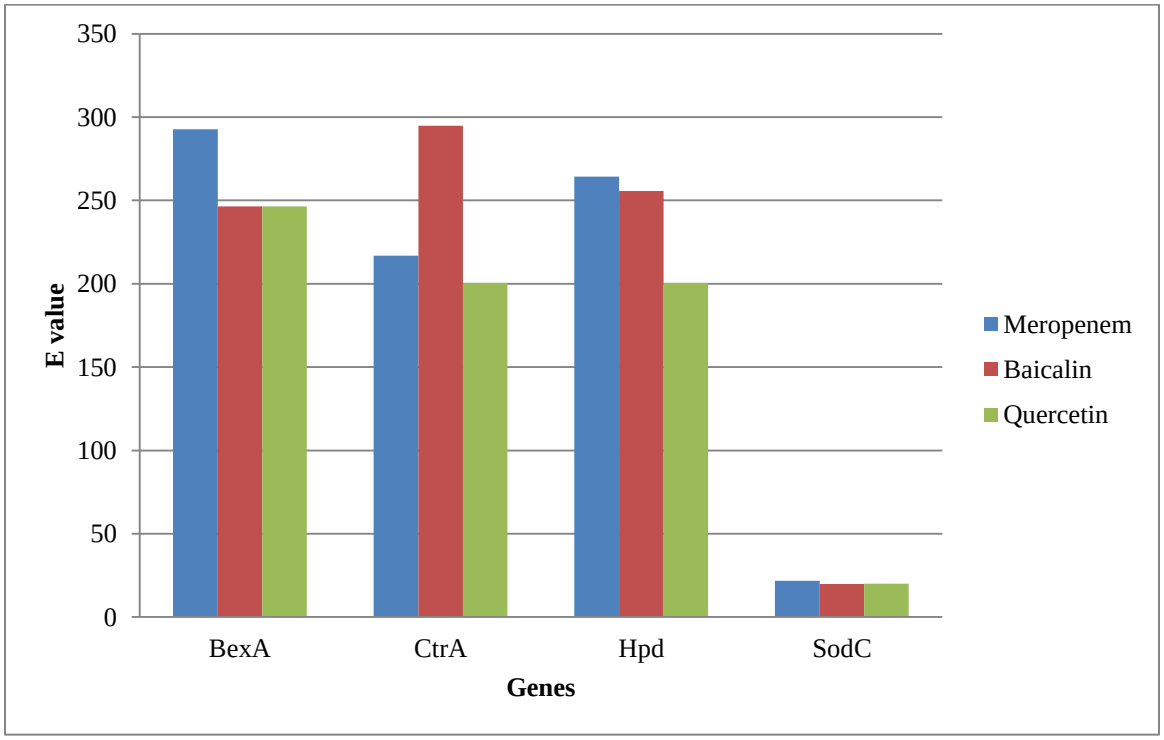


Figure-17: Docking Result of SodC with (A) Baicalin and (B) Quercetin

Protein	E. value of Meropenem	E. value of Baicalin	E. value of Quercetin
BexA	-292.78	-246.4	-246.4
CtrA	-216.82	-294.83	-200.54
HPD	-264.38	-255.67	-200.54
SodC	-21.73	-19.9	-20

Table-10: E. values of Different Drugs



Graph-2: Plotted on the basis of E. values

Hex tool was used for the analysis of the pockets and to identify the best site for the binding of ligand. This tool predicted the best possible site for ligand binding on the basis of E. values calculated from the 3D atomic coordinates of the protein and biomolecules. Docking result for Meropenem against BexA, CtrA, HPD and SodC -292.78, -216.82, -264.38, -21.73 respectively; for Baicalin against same proteins are found to be -246.4, -294.83, -255.67, and -19.9 respectively and for Quercetin against these proteins are found to be -246.4, -200.54, -200.54, and -20 in same sequence as mentioned above. From these data it is clear that Baicalin and Quercetin may work against bacterial meningitis like Meropenem.

DISCUSSION

DISCUSSION

Meningitis is a chronic neurodegenerative disorder in which inflammation of meninges occurs that results in leakage of CSF. CSF protects the brain from shock, so we termed it as a shock absorber of the brain. Meningitis could occur due to bacterial infection, viral infection or fungal infection. In bacterial meningitis *Neisseria meningitides*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b plays an important role and on targeting bexA, ctrA, HPD and SodC of these bacteria, I tried to find out the crucial role of two biomolecules i.e., Baicalin and Quercetin with help of bioinformatics tools which could be used for curing this disease. It has been experimentally checked in wet lab that Baicalin can be used for delaying the influence of meningitis and Quercetine a well-known bioflavonoid used to provide protection to neuronal cell from oxidative stress. Although the drugs to delay the influence of meningitis are available in the market but still there is no permanent treatment of this disease. More than 90% of bacterial meningitis occurs in children. So, it is still a challenging task for the scientists and doctors to identify the permanent treatment of this disease. Ramachandran plot results for BexA, CtrA, HPD and SODC displayed 96.8% of residues in the most preferred regions, 3.1% residues in additionally permitted and 0.1% in hindered regions for BexA, 95.9% of residues in the most preferred regions, 3.5% residues in additionally permitted and 0.6% in hindered regions for CtrA, 98.8% of residues in the most preferred regions, 0.8% residues in additionally permitted and 0.4% in the hindered regions for HPD and in case of SODC 96.7% of residues in the most preferred regions, 3.3% in residues in additionally permitted and no residues in hindered regions. Baicalin and Quercetin are bioflavonoids may be used for the treatment of this disease. However, both biomolecules have qualified for the rules of Lipinski filter. Dogsite Scorer and Docking results states that Baicalin and Quercetin has more druggability like Meropenem. So, it may be effective in the treatment of meningitis.

REFERENCES

REFERENCES

1. Carpenter, R. R., and R. G. Petersdorf. 1962. The clinical spectrum of bacterial meningitis. Am. J. Med. 33:262–275.
2. Hoen, B., J. F. Viel, A. Gerard, J. B. Dureux, and P. Canton. 1993. Mortality in pneumococcal meningitis: a multivariate analysis of prognostic factors. Eur. J. Med. 2:28–32.
3. Swartz, M. N., and P. R. Dodge. 1965. Bacterial meningitis—a review of selected aspects. 1. General clinical features, special problems and unsual meningeal reactions mimicking bacterial meningitis. N. Engl. J. Med. 272:842–848.
4. Scarborough, M., S. B. Gordon, C. J. Whitty, N. French, Y. Njalale, A. Chitani, T. E. Peto, D. G. Lalloo, and E. E. Zijlstra. 2007. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. N. Engl. J. Med.357:2441–2450.
5. Scarborough, M., and G. E. Thwaites. 2008. The diagnosis and management of acute bacterial meningitis in resource-poor settings. Lancet Neurol. 7:637–648.
6. Feldman RA, Koehler RE, Fraser DW. Race-specific differences in bacterial meningitis deaths in the United States, 1962-1968. Am J Publ Health 1976;66:392·6.
7. Floyd RF, Federspiel CF, Schaffner W. Bacterial meningitis in urban and rural Tennessee. Am J Epidemiol 1974;99:395-407.
8. Fraser DW, Geil CC, Feldman RA. Bacterial meningitis in Bernalillo County, New Mexico: a comparison with three other American populations. Am J Epidemiol 1974; 100:29-34.
9. Wenger JD, Hightower AW, Facklam RR, Gaventa S, Broome CV, and the Bacterial Meningitis Study group. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. J Infect Dis 1990; 162: 1316-23.
10. Wotton KA, Stiver HG, Hildes JA. Meningitis in the central Arctic: a 4-year experience. Can Med Assoc J 1981;124:887-90.
11. Garpenholt, O., S. A. Silfverdal, S. Hugosson, H. Fredlund, L. Bodin, V. Romanus, and P. Olcen. 1996. The impact of Haemophilus influenzae type b vaccination in Sweden. Scand. J. Infect. Dis. 28:165–169.
12. Robbins, J. B., R. Schneerson, P. Anderson, and D. H. Smith. 1996. The 1996 Albert Lasker Medical Research Awards. Prevention of systemic infections, especially meningitis, caused by Haemophilus influenzae type b. Impact on public health and implications for other polysaccharide-based vaccines. JAMA 276:1181–1185.

13. van Alphen, L., L. Spanjaard, H. D. van der Lei, I. Schuurman, and J. Dankert. 1997. Effect of nationwide vaccination of 3-month-old infants in the Netherlands with conjugate Haemophilus influenzae type b vaccine: high efficacy and lack of herd immunity. J. Pediatr. 131:869–873.
14. Adams, W. G., K. A. Deaver, S. L. Cochi, B. D. Plikaytis, E. R. Zell, C. V. Broome, and J. D. Wenger. 1993. Decline of childhood Haemophilus influenzae type b (Hib) disease in the Hib vaccine era. JAMA 269:221–226.
15. Mendsaikhan, J., J. P. Watt, O. Mansoor, N. Suvdmaa, K. Edmond, D. J. Litt, P. Nymadawa, Y. Baoping, D. Altantsetseg, and M. Slack. 2009. Childhood bacterial meningitis in Ulaanbaatar, Mongolia, 2002–2004. Clin. Infect. Dis. 48(Suppl. 2):S141–S146.
16. Pelkonen, T., I. Roine, L. Monteiro, M. Correia, A. Pitkaranta, L. Bernardino, and H. Peltola. 2009. Risk factors for death and severe neurological sequelae in childhood bacterial meningitis in sub-Saharan Africa. Clin. Infect. Dis. 48:1107–1110.
17. Adegbola, R. A., O. Secka, G. Lahai, N. Lloyd-Evans, A. Njie, S. Usen, C. Oluwalana, S. Obaro, M. Weber, T. Corrah, K. Mulholland, K. McAdam, B. Greenwood, and P. J. Milligan. 2005. Elimination of Haemophilus influenzae type b (Hib) disease from the Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. Lancet 366:144–150.
18. Cowgill, K. D., M. Ndiritu, J. Nyiro, M. P. Slack, S. Chiphatsi, A. Ismail, T. Kamau, I. Mwangi, M. English, C. R. Newton, D. R. Feikin, and J. A. Scott. 2006. Effectiveness of Haemophilus influenzae type b conjugate vaccine introduction into routine childhood immunization in Kenya. JAMA 296:671–678.
19. Daza, P., R. Banda, K. Misoya, A. Katsulukuta, B. D. Gessner, R. Katsande, B. R. Mhlanga, J. E. Mueller, C. B. Nelson, A. Phiri, E. M. Molyneux, and M. E. Molyneux. 2006. The impact of routine infant immunization with Haemophilus influenzae type b conjugate vaccine in Malawi, a country with high human immunodeficiency virus prevalence. Vaccine 24:6232–6239.
20. Gessner, B. D., A. Sutanto, M. Linehan, I. G. Djelantik, T. Fletcher, I. K. Gerudug, Ingerani, D. Mercer, V. Moniaga, L. H. Moulton, K. Mulholland, C. Nelson, S. Soemohardjo, M. Steinhoff, A. Widjaya, P. Stoeckel, J. Maynard, and S. Arjoso. 2005. Incidences of vaccine-preventable Haemophilus influenzae type b pneumonia and meningitis in Indonesian children: hamlet-randomised vaccine-probe trial. Lancet 365:43–52.
21. Arda, B., O. R. Sipahi, S. Atalay, and S. Ulusoy. 2008. Pooled analysis of 2,408 cases of acute adult purulent meningitis from Turkey. Med. Princ. Pract. 17:76–79.

22. Thigpen, M. C., N. E. Rosenstein, C. G. Whitney, R. Lynfield, M. M. Farley, A. S. Craig, J. Hadler, L. H. Harrison, K. Gershman, N. M. Bennett, A. R. Thomas, A. Reingold, and A. Schuchat. 2005. Bacterial meningitis in the United States—1998-2003, abstr. 1171. Abstr. 43rd Annu. Meet. Infect. Dis. Soc. Am., San Francisco, CA.
23. van de Beek, D., J. de Gans, L. Spanjaard, M. Weisfelt, J. B. Reitsma, and M. Vermeulen. 2004. Clinical features and prognostic factors in adults with bacterial meningitis. N. Engl. J. Med. 351:1849–1859.
24. Black, S., H. Shinefield, B. Fireman, E. Lewis, P. Ray, J. R. Hansen, L. Elvin, K. M. Ensor, J. Hackell, G. Siber, F. Malinoski, D. Madore, I. Chang, R. Kohberger, W. Watson, R. Austrian, and K. Edwards. 2000. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr. Infect. Dis. J. 19:187–195.
25. Tsai, C. J., M. R. Griffin, J. P. Nuorti, and C. G. Grijalva. 2008. Changing epidemiology of pneumococcal meningitis after the introduction of pneumococcal conjugate vaccine in the United States. Clin. Infect. Dis. 46:1664–1672.
26. Hsu, H. E., K. A. Shutt, M. R. Moore, B. W. Beall, N. M. Bennett, A. S. Craig, M. M. Farley, J. H. Jorgensen, C. A. Lexau, S. Petit, A. Reingold, W. Schaffner, A. Thomas, C. G. Whitney, and L. H. Harrison. 2009. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. N. Engl. J. Med. 360:244–256.
27. Casado-Flores, J., C. Rodrigo, J. Aristegui, J. M. Martinon, A. Fenoll, and C. Mendez. 2008. Decline in pneumococcal meningitis in Spain after introduction of the heptavalent pneumococcal conjugate vaccine. Pediatr. Infect. Dis. J. 27:1020–1022.
28. Greenberg, D. 2009. The shifting dynamics of pneumococcal invasive disease after the introduction of the pneumococcal 7-valent conjugated vaccine: toward the new pneumococcal conjugated vaccines. Clin. Infect. Dis.49:213–215.
29. Centers for Disease Control and Prevention. 2009. Active bacterial core surveillance. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/abcs/survreports/mening08.pdf.
30. Moore, P. S. 1992. Meningococcal meningitis in sub-Saharan Africa: a model for the epidemic process. Clin. Infect. Dis. 14:515–525.
31. Moore, P. S., M. W. Reeves, B. Schwartz, B. G. Gellin, and C. V. Broome. 1989. Intercontinental spread of an epidemic group A Neisseria meningitidis strain. Lancet ii:260–263.

32. Pinner, R. W., B. G. Gellin, W. F. Bibb, C. N. Baker, R. Weaver, S. B. Hunter, S. H. Waterman, L. F. Mocca, C. E. Frasch, and C. V. Broome. 1991. Meningococcal disease in the United States—1986. Meningococcal Disease Study Group. J. Infect. Dis. 164:368–374.
33. Boisier, P., P. Nicolas, S. Djibo, M. K. Taha, I. Jeanne, H. B. Mainassara, B. Tenebray, K. K. Kairo, D. Giorgini, and S. Chanteau. 2007. Meningococcal meningitis: unprecedented incidence of serogroup X-related cases in 2006 in Niger. Clin. Infect. Dis. 44:657–663.
34. Gardner, P. 2006. Clinical practice. Prevention of meningococcal disease. N. Engl. J. Med. 355:1466–1473.
35. Snape, M. D., and A. J. Pollard. 2005. Meningococcal polysaccharideprotein conjugate vaccines. Lancet Infect. Dis. 5:21–30.
36. Ramsay, M. E., N. Andrews, E. B. Kaczmarski, and E. Miller. 2001. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. Lancet 357:195–196.
37. Miller, E., D. Salisbury, and M. Ramsay. 2001. Planning, registration, and implementation of an immunization campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 20(Suppl.1):S58–S67.
38. Bose, A., P. Coen, J. Tully, R. Viner, and R. Booy. 2003. Effectiveness of meningococcal C conjugate vaccine in teenagers in England. Lancet 361:675–676.
39. Maiden, M. C., and J. M. Stuart. 2002. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. Lancet 359:1829–1831.
40. Maiden, M. C., A. B. Ibarz-Pavon, R. Urwin, S. J. Gray, N. J. Andrews, S. C. Clarke, A. M. Walker, M. R. Evans, J. S. Kroll, K. R. Neal, D. A. Ala'aldeen, D. W. Crook, K. Cann, S. Harrison, R. Cunningham, D. Baxter, E. Kaczmarski, J. Maclennan, J. C. Cameron, and J. M. Stuart. 2008. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. J. Infect. Dis. 197:737–743.
41. Bilukha, O. O., and N. Rosenstein. 2005. Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 54(RR7): 1–21.
42. Centers for Disease Control and Prevention. 2007. Revised recommendations of the Advisory Committee on Immunization Practices to vaccinate all persons aged 11–18 years with meningococcal conjugate vaccine. MMWR Morb. Mortal. Wkly. Rep. 56:794–795.
43. Centers for Disease Control and Prevention. 2009. Updated recommendation from the Advisory Committee on Immunization Practices (ACIP) for revaccination of persons at

prolonged increased risk for meningococcal disease. MMWR Morb. Mortal. Wkly. Rep. 58:1042–1043.

44. Plotkin, S. A., and S. L. Kaplan. 2006. Meningococcal control in the United States and Africa. J. Infect. Dis. 193:754–755.
45. Vu, D. M., J. A. Welsch, P. Zuno-Mitchell, J. V. la Cruz, and D. M. Granoff. 2006. Antibody persistence 3 years after immunization of adolescents with quadrivalent meningococcal conjugate vaccine. J. Infect. Dis. 193:821–828.
46. Ellis and Hartl (1999) Principles of protein folding in the cellular environment. Curr. Opin. Struct. Biol. 9(1): 102-110.
47. Ellis (1987) Proteins as molecular chaperones. Nature 328(6129): 378-379.
48. Ellis and van der Vies (1991) Molecular Chaperones. Annu. Rev.Biochem. 60: 321-347.
49. Hartl (1995) Principles of chaperone-mediated protein folding. Phil. Trans. R. Soc. Lond. B 348(1323): 107-112.
50. Parsell and Lindquist (1993) The Function of Heat-Shock Proteins in Stress Tolerance: Degradation and Reactivation of Damaged Proteins. Annu. Rev. Genet. 27: 437-496.
51. Gething and Sambrook (1992) Protein folding in the cell. Nature 355(6355): 33-45.
52. Ritossa (1962) New puffing pattern induced by temperature shock and dinitrophenol in Drosophila. Experientia 18: 571-573.
53. Ritossa (1996) Discovery of the heat shock response. Cell Stress Chaperon. 1(2): 97-98.
54. Tissières, Mitchell and Tracy (1974) Protein Synthesis in Salivary Glands of Drosophila melanogaster: Relation to Chromosome Puffs. J. Mol. Biol. 84(3): 389-398.
55. Ewalt, Hendrick, Houry and Hartl (1997) In Vivo Observation of Polypeptide Flux through the Bacterial Chaperonin System. Cell 90(3):491-500.
56. Hendrix (1979) Purification and Properties of groE, a Host Protein Involved in Bacteriophage Assembly. J. Mol. Biol. 129(3): 375-392.
57. Hohn, Hohn, Engel and Wurtz (1979) Isolation and Characterization of the Host Protein groE Involved in Bacteriophage Lambda Assembly. J.Mol. Biol. 129(3): 359-373.
58. Laminet, Ziegelhoffer, Georgopoulos and Plückthun (1990) The Escherichia coli heat shock proteins GroEL and GroES modulate the folding of the β-lactamase precursor. EMBO J. 9(7):
59. Chen, Roseman, Hunter, Wood, Burston, Ranson, Clarke and Saibil (1994) Location of a folding protein and shape changes in GorEL-GroES complexes images by cryo-electron microscopy. Nature 71(6494): 261-264.

60. Martin, Langer, Boteva, Schramel, Horwich and Hartl (1991) Chaperonin mediated protein folding at the surface of GroEL through a 'molten globule'-like intermediate. Nature 352(6330): 36-42.
61. Fenton, Kashi, Furtak and Horwich (1994) Residues in chaperonin GroEL required for polypeptide binding and release. Nature 371(3498): 614-619.
62. Ishii, Taguchi, Sasabe and Yoshida (1994) Folding Intermediate Binds to the Bottom of Bullet-shaped Holo-chaperonin and is Readily Accessible to Antibody. J. Mol. Biol. 236(3): 691-696.
63. Chandrasekhar, Tilly, Woolford, Hendrix and Georgopoulos (1986) Purification and Properties of the groES Morphogenetic Protein of Escherichia coli. J. Biol. Chem. 261(26): 12414-12419.
64. Langer, Pfeifer, Martin, Baumeister and Hartl (1992) Chaperonin mediated protein folding: GroES binds to one end of the GroEL cylinder, which accommodates the protein substrate within its central cavity. EMBO J. 11(13): 4757-4765.
65. Braig, Simon, Furuya, Hainfeld and Horwich (1993) A polypeptide bound by the chaperonin groEL is localized within a central cavity. 90(9): 1978-3982.
66. Martin, Mayhew, Langer and Hartl (1993). The reaction cycle of GroEL and GroES in chaperonin-assisted protein folding. Nature 366(6452):228-233.
67. Mayhew, da Silva, Martin, Erdjument-Bromage, Tempst and Hartl (1996) Protein folding in the central cavity of the GroEL-GroES chaperonin complex. Nature 379(6564): 420-426.
68. Mogk, Mayer and Deuerling (2002) Mechanisms of Protein Folding: Molecular Chaperones and Their Application in Biotechnology. ChemBioChem 3(9): 807-814.
69. Landry, Jordan, McMacken and Gierasch (1992) Different conformations for the same polypeptide bound to chaperones DnaK and GroEL. Nature 355(6359): 455-547.
70. Fourie, Sambrook and Gething (1994) Common and Divergent Peptide Binding Specificities of hsp70 Molecular Chaperones. J. Biol. Chem. 269(48): 30470-30478.
71. Rüdiger, Germeroth, Schneider-Mergener and Bukau (1997) Substrate specificity of the DnaK chaperone determined by screening cellulosebound peptide libraries. EMBO J. 16(7): 1501-1507.
72. Rüdiger, Schneider-Mergener and Bukau (2001) Its substrate specificity characterizes the DnaJ co-chaperone as a scanning factor for the DnaK chaperone. EMBO J. 20(5): 1042-1050.
73. Beckmann, Welch and Mizzen (1990) Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science 248(4957): 850-854.
74. Flynn, Chappell and Rothman (1989). Peptide binding and release by proteins implicated as catalysts of protein assembly. Science 245(4916):385-390.

75. Langer, Lu, Echols, Flanagan, Hayer and Hartl (1992). Successive action of DnaK, DnaK and GroEL along the pathway of chaperone-mediated protein folding. Nature 356(6371): 683-689.
76. Thulasiraman, Yang and Frydman (1999). In vivo newly translated polypeptides are sequestered in a protected folding environment. EMBO J. 18(1): 85-95.
77. Buchner (1996) Supervising the fold: functional principles of molecular chaperones. FASEB J. 10(1): 10-19.
78. Neckers, Mimnaugh and Schulte (1999). The Hsp90 Chaperone Family. Handb. Exp. Pharmacol. 136 (Stress Proteins): 9-42.
79. Koyasu, Nishida, Kadowaki, Matsuzaki, Iida, Harada, Kasuga, Sakai and Yahara (1986). Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. Proc. Natl. Acad. Sci. USA 83(21): 8054-8058.
80. Minami, Kawasaki, Miyata, Suzuki and Yahara (1991) Analysis of Native Forms and Isoform Compositions of the Mouse 90-kDa Heat Shock Protein, HSP90. J. Biol. Chem. 266(16): 10099-10103.
81. Obermann, Sondermann, Russo, Pavletich and Hartl (1998) In Vivo Function of Hsp90 Is Dependent on ATP Binding and ATP Hydrolysis. J. Cell. Biol. 143(4): 901-910.
82. Richter and Buchner (2001) Hsp90: Chaperoning Signal Transduction. J. Cell. Physiol. 188(3): 188-281.
83. Pratt and Toft (2003) Regulation of Signaling Protein Function and Trafficking by the hsp90/hsp70-Based Chaperone Machinery. Exp. Biol. Med. 228(2): 111-133.
84. Picard, Khursheed, Garabedian, Fortin, Lindquist and Yamamoto (1990). Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature 348(6297): 166-168.
85. Pearl and Prodromou (2002) Structure, Function, and Mechanism of the Hsp90 Molecular Chaperone. Adv. Protein Chem. 59 (Protein Folding in the Cell): 157-186.
86. Morishima, Murphy, Li, Sanchez and Pratt (2000) Stepwise Assembly of a Glucocorticoid Receptor—hsp90 Heterocomplex Resolves Two Sequential ATP-dependent Events Involving First hsp70 and Then hsp90 in Opening of the Steroid Binding Pocket. J. Biol. Chem. 275(24): 18054-18060.
87. Jakob and Buchner (1994) Assisting spontaneity: the role of Hsp90 and small Hsps as molecular chaperones. TIBS 19(5): 205-211.
88. Young, Moarefi and Hartl (2001) Hsp90: a specialized but essential protein-folding tool. J. Cell. Biol. 154(2): 267-273.

89. Farley MM, Stephens DS, Mulks MH. Pathogenesis of IgA1 protease-producing and nonproducing Haemophilus injluenzae in human nasopharyngeal organ cultures. J Infect Dis 1986;154:752-9.
90. Plaut AG. The IgAI proteases of pathogenic bacteria. Annu Rev Microbiol 1983;37:603-22.
91. Stephens DS, Farley MM. Pathogenic events during infection of the human nasopharynx with *Neisseria meningitidis* and *Haemophilus influenzae.* Rev Infect Dis 1991;13:22-33.
92. Stephens DS, Hoffman LH, McGee ZA. Interaction of Neisseria meningitidis with human nasopharyngeal mucosa: Attachment and entry into columnar epithelial cells. J Infect Dis 1983;148:369-76.
93. Lynn WA, Raetz CRH, Qureshi N, Golenbock DT. Lipopolysaccharide-induced stimulation of CD1l/CD18 expression on neutrophils. Evidence of specific receptor-based response and inhibition by lipid A-based antagonists. J Immunol 1991; 147:3072-9.
94. Moser R, Schleiffenbaum B, Groscurth P, Fehr J. Interleukin 1 and tumor necrosis factor stimulate human vascular endothelial cells to promote transendothelial neutrophil passage. J Clin Invest 1989;83:444-55.
95. Pober JS, Cotran RS. Cytokines and endothelial cell biology. Physiol Rev 1990;70:427-51.
96. Pober JS, LaPierre LA, Stolpen AH, et al. Activation of cultured endothelial cells by recombinant Iymphotoxin: comparison with tumor necrosis factor and interleukin-l species. J Immunol 1987;138:3319-24.
97. Rothlein R, Czaikowski M, O'Neil MM, Martin D, Mainolfi E, Merluzzi J. Induction of intercellular adhesion molecule-1 on primary and continuous cell lines by proinflammatory cytokines. J Immunol 1988; 141:1665-9.
98. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-l/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989;84:1045-49.
99. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068-101.
100. Springer TA. Adhesion receptors of the immune system. Nature 1980;346:425•434.
101. Tureen JH, Tauber MG, Sande MA. Effect of hydration status on cerebral blood flow and cerebrospinal fluid lactic acidosis in rabbits with experimental meningitis. J Clin Invest 1992;89:947-53.
102. Cooper A, Beaty H, Oppenheimer S, Goodner R, Petersdorf R. Studies on the pathogenesis of meningitis. Glucose transport and spinal fluid production in experimental pneumococcal meningitis. J Lab Clin Med 1968;71:473-83.

103. Prockop L, Fishman R. Pathophysiology of the cerebrospinal fluid changes in experimental pneumococcal meningitis. Trans Am Neurol Assoc 1966;91:126-31.
104. Guerra-Romero L, Tauber MG, Fournier MA, Tureen JH. Lactate and glucose concentrations in brain interstitial fluid, cerebrospinal fluid, and serum during experimental pneumococcal meningitis. J Infect Dis 1992;166:546-50.
105. Scheld WM. Morphofunctional alterations of the blood-brain barrier during experimental meningitis. Pediatr Infect Dis J 1987;6:1145-6.
106. Machka K, Braveny I, Dabernat H, et al. Distribution and resistance patterns of Haemophilus influenzae: a European cooperative study. Eur J Clin Microbiol Infect Dis 1988;7:14-24.
107. Campos J, Garcia-Tornel S, Sanfeliu I. Susceptibility studies of multiply resistant Haemophilus influenzae isolated from pediatric patients and contacts. Antimicrob Agents Chemothcr 1984;25:706-9.
108. Campos J, Garcia-TorneI S. Comparative susceptibilities of ampicillin and chloramphenicol resistant Haemophilus influenzae to fifteen antibiotics. J Antimicrob Chemother 1987;19:297-301.
109. Garvey RJP, McMuliin GP. Meningitis due to β-lactamase producing type b Haemophilus influenzae resistant to chloramphenicol. BMJ 1983;287:1183-84.
110. de Groot R, Dzoljic-Danilovic G, van Klingeren B, Goessens WH, Neyens HJ. Antibiotic resistance in Haemophilus influenzae: mechanisms, clinical importance and consequences for therapy. Eur J Pediatr 1991;150:534-46.
111. Simasathien S, Duangmani C, Echeverria P. Haemophilus influenzae type b resistant to ampicillin and chloramphenicol in an orphanage in Thailand. Lancet 1980;2:1214-7.
112. Uchiyama N, Greene GR, Kitts DB, Thrupp LD. Meningitis due to Haemophilus influenzae type b resistant to ampicillin and chloramphenicol. J Pediatr 1980;97:421-4.
113. Saez-Nieto JA, Lujan R, Berron, et al. Epidemiology and molecular basis of penicillin resistant Neisseria meningitidis in Spain: a 5-year history (1985-1989) Clin Infect Dis 1992;14:394-402.
114. Cherubin CE, Eng RHK, Norrby R, Modai J, Humbert G, Overturf G. Penetration of newer cephalosporins into cerespinaI fluid. Rev Infect Dis 1989;11:526-48.
115. Klein NJ, Heyderman RS, Levin M. Antibiotic choices for meningitis beyond the neonatal period. Arch Dis Child 1992;67:157-61.
116. Neu HC. Cephalosporins in the treatment of meningitis. Drugs 1987;34[Suppl]:135-53.
117. Steele RW. Cephalosporins for bacterial meningitis: which one is best? J Pediatr 1989;114:991-2.

118. Fong IW, Tomkins KB. Penetration of ceftazidime into the cerebrospinal fluid of patients with and without evidence of meningeal inflammation. Antimicrob Agents Chemother 1984;26:115-6.
119. Hatch D, Overturf GD, Kovacs A, Forthal D, Leong C. Treatment of bacterial meningitis with ceftazidime. Pediatr Infect Dis J 1986;5:416-20.
120. Rodriguez WJ, Puig JR, Khan WN, Feris J, Gold BG, Sturla C. Ceftazidime vs standard therapy for pediatric meningitis: therapeutic. pharmacologic and epidemiologic observations. Pediatr Infect Dis 1986;5:408-15.
121. Radetsky M. Duration of treatment in bacterial meningitis: a historical inquiry. Pediatr Infect Dis J 1990;9:2-9.
122. Felgin RD. Bacterial meningitis beyond the newborn period. In: Feigin RD, Cherry JD (eds) Textbook of pediatric infectious diseases. Saunders, Philadelphia, 1987:439-65.
123. Kaplan SL, Feigin RD. The syndrome of inappropriate secretion of antidiuretic hormone in children with bacterial meningitis. J Pediatr 1978;92:758-61.
124. Powell KR, Sugerman LI, Eskenazi AE, et al. Normalization of plasma arginine vasopressin concentrations when children with meningitis are given maintenance plus replacement f1uid therapy. J Pediatr 1990;117:515-22.
125. Tauber MG, Sande E, Fournier MA, Tureen JH, Sande MA. Fluid administration, brain edema, and cerebrospinal fluid lactate and glucose concentrations in experimental Escherichia coli meningitis. J Infect Dis 1993;168:473-6.
126. Clemens JD, Ferreccio C, Levine MM, et al. Impact of Haemophilus influenzae type b polysaccharide-tetanus protein conjugate vaccine on responses to concurrently administered diphtheria-tetanus-pertussis vaccine. JAMA 1992;267:673-8.
127. Eskola J, Peltola H, Takala AK, et al. Efficacy of Haemophilus influenzae type b polysaccharide-diphtheria toxoid conjugate vaccine in infancy. N Engl J Med 1987;317:717-22.
128. Eskola J, Kayhty H, Takala AK, et al. A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive Haemophilus injluenzae type b disease. N Engl J Med 1990;323:1381-7.
129. Granoff DM, Cates KL. Haemophilus injluenzae type b polysaccharide vaccines. J Pediatr 1985; 107:330-6.

130. Granoff DM, Anderson EL, Osterhohn MT, Holmes SJ, McHugh JE, Belshe RB, et al. Differences in the immunogenicity of three Haemophilus injluenzae type b conjugate vaccines in infants. J Pediatr 1992;121:187-94.
131. Kayhty H, Eskola J, Peltola H, et al. Antibody responses to four Haemophilus injluenzae type b conjugate vaccines. JAMA 1991;145:223-7.
132. Peltola H, Kayhty H, Virtanen M, Makela PH. Prevention of Haemophilus injluenzae type b bacteremic infections with the capsular polysaccharide vaccine. N Engl J Med 1984;310:1561-6.
133. Watemberg N, Dagan R, Arbelli Y, et al. Safety and immunogenicity of Haemophilus injluenzae type b-tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus-pertussis vaccine in young infants. Pediatr Infect Dis J 1991;l0:758-61.
134. Peltola H, Kilpi T, Anttila M. Rapid disappearance of Haemophilus injluenzae type b meningitis after routine childhood immunisation with conjugate vaccines. Lancet 1992;340:592-4.
135. Frasch CE. Vaccines for prevention of meningoccocal disease. Clin Microbiol Rev 1989;2:S134-8.
136. van der Ley P, van der Biezen J, Hohenstein P, Peeters C, Poolman JT. Use of transformation to construct antigenic hybrids of the class 1 outer membrane protein in Neisseria meningitidis. Infect Immun 1993;61:4217-24.
137. Bjune G, Hoiby EA, Gronnesby JK, ef al. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet 1991;1093-6.
138. Cassio de Moraes J, Perkins BA, Camargo MCC, et al. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. Lancet 1992;340:1074-8.
139. Steinhoff MC, Edwards K, Keyserling H, et al. A randomized comparison of three bivalent Streptococcus pneumoniae glycoprotein conjugate vaccines in young children: effect of polysaccharide size and linkage characteristics. Pediatr Infect Dis J 1994; 13:368-72.