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Prevalence, *In Silico* Analysis and Possible Molecular Drug Therapy for Specific Meningitis Types

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ABSTRACT

Background. Meningitis is the inflammation of the membranes surrounding the brain and spinal cord. This study was designed to determine the prevalence of meningitis along with the docking of bacterial proteins with drugs used to treat meningitis.

Methodology. A cross-sectional study was conducted including 100 febrile seizure children admitted to the hospitals of Hyderabad, Pakistan. Demographic data was recorded via a questionnaire. Cerebrospinal fluid (CSF) and blood were analyzed clinically. Molecular docking via PyRx—a virtual screening tool—was performed and Chi-square test was applied to interpret the data.

Results. Out of the 100 patients included in the study, 32 had been diagnosed with meningitis, 53 were suspected patients, and 15 were patients of encephalitis. Among diagnosed meningitis patients, 47% had bacterial meningitis, 16% had viral meningitis, while only 9% had TBM. Moreover, 28% of patients were partially treated with a mortality rate of 18%. Low glucose levels, high protein levels, and high neutrophil count were found in cerebrospinal fluid (CSF). The majority of patients were vaccinated (48%). Meningitis was more frequent among infant children and the results were statistically significant (p-value = 0.01). Molecular docking revealed that ceftriaxone had the greatest affinity for bacterial proteins, while Haemophilus influenza Hia Adhesin (3syj) and Pneumolysin (5aoe) had the lowest Kd values of -9 and -8.6, respectively.

Conclusion. Bacterial meningitis was found to be the most prevalent type with 32% prevalence. Ceftriaxone could be the drug of choice if meningitis is caused by 3syj or 5aoe. However, either vancomycin or meropenem may be preferred over ceftriaxone if meningitis is caused by 1p4t (Neisserial surface protein A).

Keywords: cerebrospinal fluid (CSF), ceftriaxone, meningitis, molecular docking, meropenum. vancomycin

Highlights

- Infants (0-2 years) are more susceptible to meningitis.
- The majority of patients were found to be affected by bacterial meningitis, followed by viral and tuberculosis meningitis, with 18% mortality rate.

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- Ceftriaxone showed highest affinity against Haemophilus influenza Hia Adhesin (3syj) and Pneumolysin (5aoe), with lowest Kd values as compared to vancomycin and meropenum
- Ceftriaxone is predictably the drug of choice if meningitis is caused by bacterial proteins 3syj or 5aoe.

1. INTRODUCTION

Seizures are documented as the cause of 1-5% of visits to emergency departments, especially in the case of pediatric disorders. Febrile seizures (FS) can occur during the age of 6-60 months. These are known to be the most frequent childhood seizures with a peak age between 1-3 years [1]. These seizures are accompanied with fever and fits. Meningitis is characterized by the inflammation of membranes or fluids surrounding the brain or spinal cord. Literature shows a connection between meningitis and febrile seizures. In most meningitis patients, seizures have been reported to be the earliest indication of meningeal infection [2]. The mortality rate of bacterial meningitis increases and changes depending on factors such as location, time, age, and gender [3]. High mortality and morbidity rates are commonly found in underdeveloped countries. The three most prevalent pathogens causing meningitis are *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumonia*, which have been reported to be responsible for over 80% of cases. However, less prevalent are group b streptococcus and *Mycobacterium tuberculosis* (M.tb) [4]**.** They enter blood from the nose, escaping the blood-brain barrier. They produce immune response upon the inflow of WBCs into CSF. The presence of leukocytosis in CSF is a major indicator of bacterial meningitis, since they feed on glucose lowering its concentration [5]. A protein level of greater than 200 mg/dl is considered significant for bacterial meningitis, as it indicates the disruption of the blood-CSF barrier [3]. The clinical symptoms of meningitis include fever as high as 99.5°F, headache, vomiting, and meningismus (a triad of 3 symptoms syndrome including neck stiffness, photophobia, and headache) [6]. Correct diagnosis requires lab testing. Different serotypes cannot be distinguished and treatment for a specific pathogen doesn't exist either. To resolve this issue, molecular docking can be applied to predict the specific drug for the specific serotype. Docking also helps to understand the bonding type, binding affinity, and interaction mode of drugs with microbial proteins.

The current study aims to find out the incidence of meningitis in children admitted with fever and fits, as well as the effects of age, gender, residence, and vaccination status on the prevalence of meningitis. It also aims to determine the interaction mode and binding affinity of the most frequently used antibiotics with specific bacterial strains and their specific proteins in order to design new and effective drugs against the disease. For molecular docking analysis, Pneumolysin (5aoe), Niesserial surface protein A(1p4t), Haemophilus influenza Hia Adhesion (3syj), and alpha C protein (1ywm) were selected. These bacterial proteins were docked with various ligands (drug) such as ceftriaxone, vancomycin, and meropenem using PyRx virtual screening tool.

2. MATERIALS AND METHODS

The current cross-sectional study included 100 febrile seizures children, from newborns to 10-year olds, who were admitted to LUHMS Hospital Hyderabad with symptoms such as neck stiffness, fever, and fits. Any cases with coma, brain tumor, and other neurological disorders were excluded from the study. The patients were monitored on an hourly basis and their vitals (temperature, heart rate, respiratory rate) were checked after every 3-4 hours.

The study was conducted with the approval of the ethical committee of the Institute of Biochemistry, University of Sindh (Jamshoro) (Date: 09.06.2023, Ref. number: IOB/368/2023). A questionnaire was designed to record the demographics of the patients including their age, gender, and residence. Vaccination status was also assessed, that is, if they had been vaccinated first or multiple times. Home-based prophylaxis, taken at the time of prodromal symptoms, was recorded. Brudzinski's sign and Kernig's sign were checked which indicated the presence of meningitis by neck stiffness and involuntary bending of knees and hips. CSF was collected with spinal needle from the subarachnoid spaces. In this regard, 1-5 ml of CSF sample was collected from each patient for physical, chemical, and microscopic examination. Oral and written permission was taken from parents/guardians before the collection of CSF samples. CSF collected through lumber puncture was evaluated for color, volume, turbidity, and chemical tests including protein, glucose, and WBCs, specifically. Blood sample was assessed to examine RBCs, WBCs, hemoglobin, hematocrit, and platelets.

Docking was executed to predict the possible mechanism of meningitis. Moreover, 3D structures of bacterial proteins were obtained via PDB (protein data bank). These included Pneumolysin (5aoe), Niesserial surface protein A (1p4t), Haemophilus influenza Hia Adhesion (3syj), and alpha C protein (1ywm). Pubchem was used to obtain the structures of ligands (ceftriaxone, vancomycin, and meropenem). Ligands were downloaded in 2D-SDF format, while 3D protein structures were downloaded in PDB format. Hetero atoms and water groups were removed using discovery visualizer. The PyRx virtual screening tool was used to calculate binding affinities which were saved as CSV files. Protein ligand interactions were identified with the help of discovery studio. Whereas, 2D interactions and 3D interactions were saved as JPG images.

For statistical analysis, frequency and percentage were calculated for age and gender. Chi-square analysis was done using SPSS to find out the association of various factors with meningitis, with a p-value of < 0.05 considered as significant.

3. RESULTS

A total of 100 patients were incorporated in this study, out of which 32 were diagnosed with different types of meningitis. Moreover, 53 patients showed physical signs and symptoms of the disease but their parents didn't allow the lumber puncture, so their meningitis status was reported as suspected (Table 1).

Table 1. Frequency Distribution of Different Factors Contributing to Meningitis

Variable	Percentage (%)
Age (months)	
0-24	56
25-36	9
37-60	13
>60	22

Variable	Percentage (%)
Area	
Urban	14
Rural	86
Gender	
Male	57
Female	43
Fatality	
Alive	82
Dead	18
Clinical features	
Fits	95
Fever	93
Cough	10
Neck stiffness	5
Unconscious	15
Vomiting	10
Lumbar puncture	
Done	32
Not done	68
Immunization	
Vaccinated	48
Non vaccinated	35
Partially vaccinated	17
Meningitis	
Diagnosed	32
Suspected	53
Encephalitis	15
Bacterial	15
Viral	5
TBM	3
Partially treated	9
Duration of fits	
Less than 15 mins	64
More than 15 mins	36
Drugs	
Ceftriaxone	95
Vancomycin	57
Meropenem	5

The remaining 15 patients were diagnosed with encephalitis, which is not categorized under the term meningitis. Therefore, the prevalence of confirmed meningitis cases in this study was 32%.

Out of the 100 patients included in the study, 56% were in the age range of 0-24 months, 22% were in the age range of 25-60 months, and 22% were older than 60 months. Further, 86% of affected patients belonged to rural areas and the ratio of boys (57%) was higher as compared to girls (43%).

The case fatality rate in this study was found to be 18% (18/100) in children having fits and fever, whereas it was 9% (3/32) in children diagnosed with meningitis. Furthermore, 95% patients suffered from fever and fits, although lumbar puncture was done only in 32% of cases. An elevated ratio of suspected meningitis (53%) was observed in the current study due to hesitation in performing the lumbar puncture. Surprisingly, the ratio of vaccinated patients was higher (48%) than non-vaccinated (35%) or partially vaccinated (17%) patients. Out of 100 patients, 64 were admitted with fits (<15 min duration), whereas 36 with fits (>15 min duration). Most patients were given ceftriaxone along with vancomycin, whereas only 5 were treated with meropenem. Therefore, ceftriaxone was established as the drug of choice for meningitis treatment. Out of the 32 children diagnosed with the disease, 15 (47%) were diagnosed with bacterial, 5 (16%) with viral, and 3 (9%) with TBM, while 9 (28%) patients were reported as partially treated (All results are shown in Table 1).

Patients diagnosed with the disease showed low CSF glucose level and high protein level in their CSF DR, which is an indication of bacterial meningitis (Table 2). While identifying the blood parameters via CBC, a higher neutrophil value (>80%) was seen as an indication of bacterial meningitis (Table 2). Chi square test was performed to find out the association of various factors

with the disease. No statistical significance was observed between gender and fits duration. The disease was found to be more prevalent among infant children with statistical significance (*p*-value = 0.01) (Figure 1). Conversely, vaccination status of children showed no statistical significance.

Table 2. CSF and CBC Parameters

CSF Parameters	Median	Min	Max
WBC (/cmm)	17.5	4	4000
Glucose (mg/dl)	57.5	5	178
Protein (mg/dl)	44.1	12.5	1275.4
CBC Parameters	Median	Min	Max
RBCs (10^{e12}/L)	4	2.3	6.4
Hemoglobin (g/dl)	10	4.5	23.2
Total Leukocyte Count (10^{e9} cells/L)	11.7	1	42.8
Platelet (10^{e9}/L)	360	13	776
Neutrophil (%)	65.5	30	88
Lymphocyte (%)	30	10	67

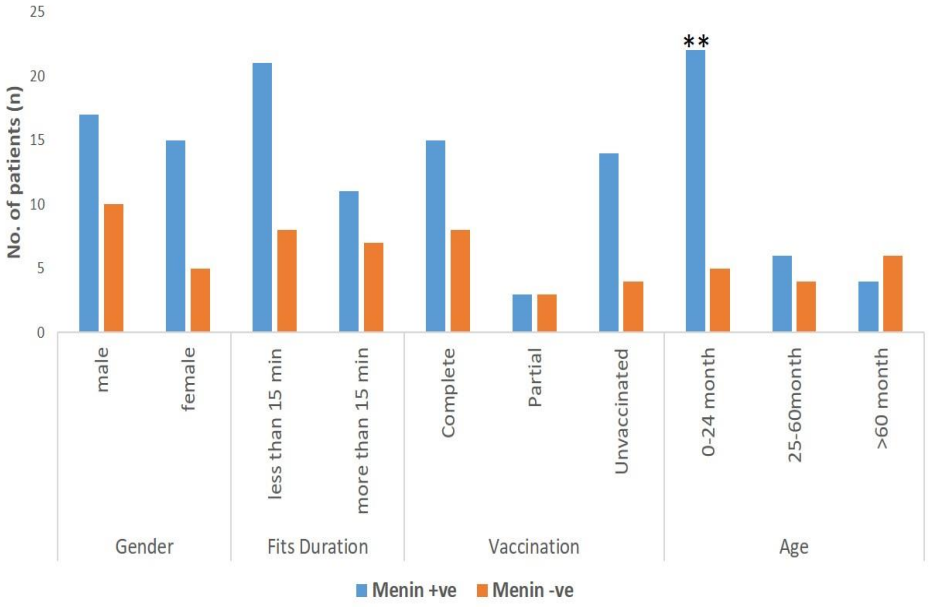

Figure 1. Statistical Association of Various Parameters with Meningitis
***p*-value < 0.05

Docking results manifested that ceftriaxone showed the greatest affinity for bacterial proteins, namely Haemophilus influenza Hia Adhesin (3syj) and Pneumolysin (5aoe), with the lowest K_d values of -9 and -8.6, respectively. The lower the K_d value, the better is the binding affinity of a ligand with its target. Binding affinity with Haemophilus influenza Hia Adhesin (3syj) and Pneumolysin (5aoe) increased in the following order: ceftriaxone > vancomycin > meropenem. Although, in the case of Neisserial surface protein A (1p4t), it increased in the

following sequence: vancomycin > meropenem > ceftriaxone (Table 3).

Table 3. Binding Affinities of Drug Interactions with Bacterial Proteins

Drugs	1p4t	5aoe	3syj	1ywm
Ceftriaxone	-5.6	-8.6	-9	-6.7
Vancomycin	-6.4	-6.4	-8.2	-5.5
Meropenem	-5.9	-5	-5.7	-5.3

The 2D diagram of Fig 2a shows that ceftriaxone interacted with bacterial protein 3syj by forming hydrogen bonds with threonine 513,493 (Figure 2A). Both ceftriaxone and vancomycin formed hydrogen bonds with arginine 501, glutamic acid 42,522, and asparagine 113,43 of that protein (Figure 2A, 3B). Vancomycin and meropenem showed the highest binding affinity for protein 1p4t, with K_d values of-6.4 and -5.9 respectively, by forming hydrogen bonds with alanine 149 and arginine 90 (Figure 3A, 4A). Ceftriaxone showed its second-highest binding affinity (-8.6) with protein 5aoe by forming a hydrogen bond with glutamic acid 35 (Figure 2B).

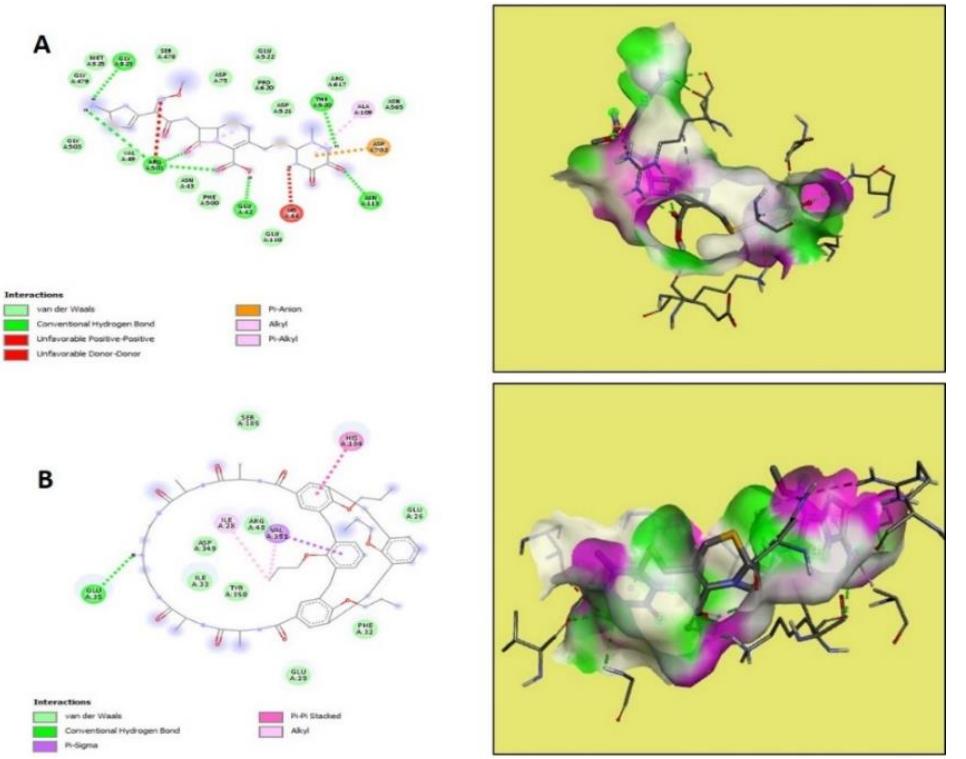

Figure 2. 2D and 3D Graphical Representation of Drug Binding Modes in Bacterial Proteins (A) Ceftriaxone with 3syj (B) Ceftriaxone with 5aoe

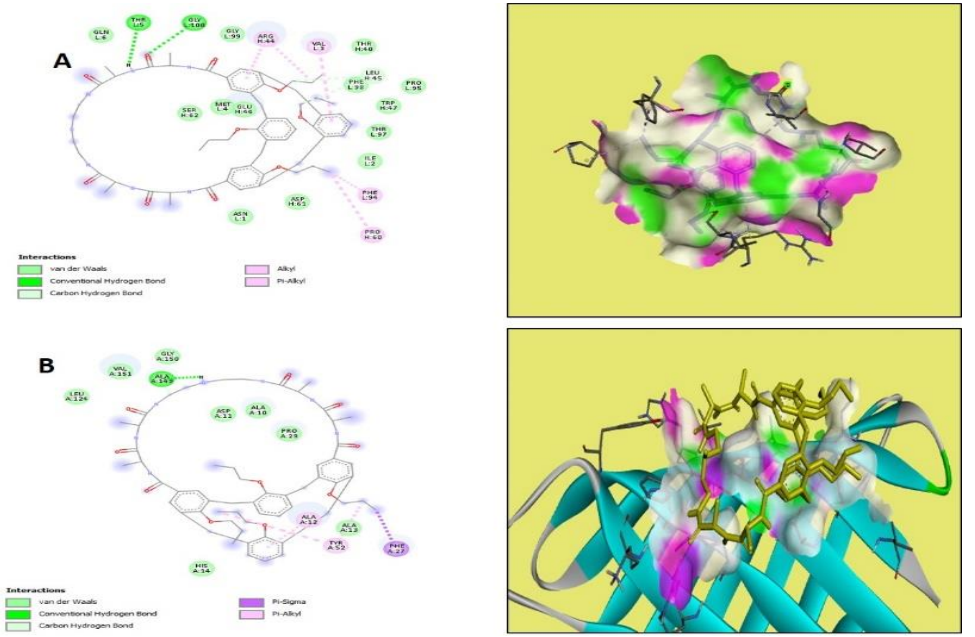

Figure 3. 2D and 3D Graphical Representation of Drug Binding Modes in Bacterial Proteins (A) Vancomycin with 1p4t (B) Vancomycin with 3syj

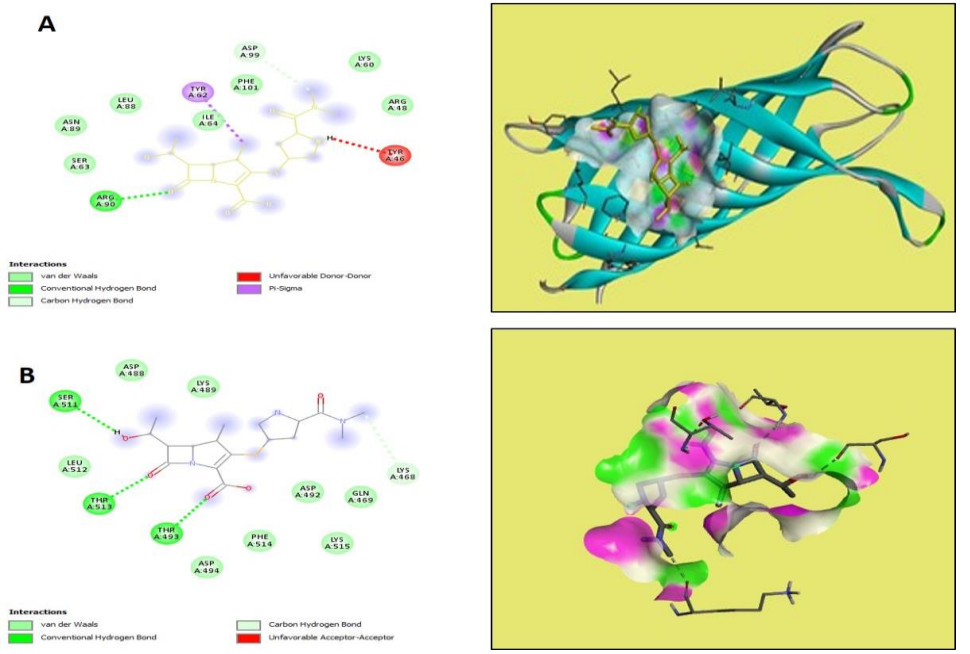

Figure 4. 2D and 3D Graphical Representation of Drug Binding Modes in Bacterial Proteins A) Meropenem with 1P4t (B) Meropenem with 3syj

4. DISCUSSION

A febrile seizure is a convulsive disorder that affects young children, usually followed by fever. A similar study was conducted in Karachi and Quetta and the cases were 19% and 38%, respectively. This study was conducted to find the incidence of meningitis in children presenting with fever and fits in Hyderabad, Pakistan. The results align with the understanding that meningitis occurs mostly in children because of their weak immunity [3, 7].

Most patients belonged to rural areas because of limited healthcare access and excessive tobacco usage in the vicinity, which causes upper respiratory tract infection, resulting in pneumonia [8]. A higher ratio of male patients indicates low immunity in them as compared to female patients [3]. This is because the estrogen hormone in women boosts type 1 T helper cell's level, which strengthens the immune system against bacterial pathogens [9]. Deaths are caused by several pathogens including *Streptococcus pneumonia* and *Mycobacterium tuberculosis.* These are deadly and known for their high mortality rate [10].

In the current study, the majority of cases were of bacterial meningitis because pathogens such as *Neisseria meningitidis*, *Haemophilus influenza*, and *Streptococcus pneumonia* are responsible for the majority of bacterial meningitis cases [11]. All of these can easily spread through the nasopharyngeal pathway and then move towards blood and meninges after several hours [12]. A higher neutrophil value (>80%) was seen as an indication of bacterial infection. During normal conditions, the central nervous system (CNS) does not have significant neutrophil immune surveillance; however, during bacterial meningitis, there is a huge mobilization of neutrophils across the blood-brain barrier aimed to eradicate the bacteria [13]. *Cryptococcus neoformans* causes the dysfunction of the blood-brain barrier and the bacteria enters CSF, where it increases glycolysis resulting in low glucose levels in the CSF of the infected patients [14]. During bacterial infection, protein level in CSF rises because of the escalated number of replicating bacteria and body cells fighting the infection, which might be the reason for the high concentration of protein. Previous studies reported higher CSF protein concentrations in bacterial meningitis patients, as compared to viral meningitis patients [15]. In a study about meningitis prevalence in Islamabad, Pakistan, CSF protein levels were also found to increase (> 80 mg/dl) in 85.5% of bacterial meningitis cases [16]. However, in the current study, out of 32 patients 31% had a higher protein level and 28% had a lower glucose level. Moreover, 95% of patients experienced fits due to complications such as edema, hydrocephalus, and increased intracranial pressure in the brain tissue [17]. Furthermore, the patients in this study were all children which made it more difficult for the family to allow lumbar puncture. The increased ratio of meningitis in vaccinated patients may be due to their vaccination only by the pentavalent vaccine (available free of cost in Pakistan), instead of the special meningococcal and pneumococcal vaccine [18].

To explore the underlying pathogenesis mechanism of meningitis, the docking interactions of commonly used drugs were analyzed against four important bacterial proteins known to cause meningitis. The lowest kd values were shown by ceftriaxone against Haemophilus influenza Hia Adhesin (3syj) and Pneumolysin (5aoe). Haemophilus influenza Hia Adhesin is a protein of the

bacteria *Haemophilus influenza*. It mediates bacterial attachment to host respiratory epithelial cells, an essential step in the process of colonization [19]. Whereas, Pneumolysin is a key player in pneumococcal meningitis, promoting bacterial invasion. However, in the case of Neisserial surface protein A (1p4t), the lowest kd values were found for vancomycin. Neisserial surface protein A (1p4t) is a meningococci that uses its surface protein known as Neisserial surface protein A (NspA) to bind to a host complement inhibitory protein, that is, factor H (fH). Enhanced complement activation occurs on the bacterial surface and increased complement-dependent killing of meningococci can be seen as a result of the loss of NspA [20]. Figures 2-4 show the interaction sites of selected drugs with amino acids of bacterial protein. Therefore, it can be inferred from the docking kd values that among all drugs, ceftriaxone might be the drug of choice if meningitis is caused by 3syj or 5aoe. However, either vancomycin or meropenem may be preferred over ceftriaxone if meningitis is caused by Neisserial surface protein A (1p4t).

5. Conclusion

The current study revealed 32% prevalence of meningitis with a 9% mortality rate. Bacterial meningitis was mostly found with higher neutrophil count, increased WBC count, low glucose levels, and elevated protein values. Age was found to be a statistically significant factor associated with meningitis, whereas gender, fits duration, and vaccination status didn't seem to affect meningitis prevalence. Molecular docking analysis suggested the use of ceftriaxone for *Haemophilus influenzae* and pneumococcal meningitis, as well as the use of vancomycin or meropenem for meningococcal meningitis. These predicted protein-drug interactions can be confirmed in future studies via molecular docking stimulations for a better understanding of the mechanism of meningitis.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

FUNDING DETAILS

No funding has been received for this research.

ETHICAL STATEMENT

This study was approved by the ethical committee of Institute of Biochemistry, University of Sindh Jamshoro. The parents of all the participants gave informed written consent prior to this study.

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