Virtual Screening and Molecular Docking Study to Identify Novel Inhibitors Against Japanese Encephalitis Virus

Kamal Soni¹, Ruchi Yadav^{1*}

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India..

ABSTRACT

Japanese encephalitis (JE) is one of the most frequent causes of viral encephalitis in humans and belongs to the family of flaviviruses (Flaviviridae). Japanese Encephalitis Virus (JEV) is transmitted by mosquitoes and vectors from other organisms, so it is also known as a mosquito-borne disease of the Japanese encephalitis virus, which includes both positive-sense ssRNA (single-stranded RNA). Includes 11 kb and several structural proteins (C, M and E) and 2 non-resistant proteins that are Non-Structural (NS) (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) of these, E protein plays an important part in immunogenicity, virus-cell membrane fusion, infection, and virus maturation. The capsid protein C plays a critical role in virus budding by attaching to the cell membrane and functions in the Nucleocapsid in the assembly of viral RNA. In this study, we have identified potential inhibitors of JEV protein. Three proteins viz. Uniprot IDs P0DOH9, P0DOK8 and G3FEX6 were selected, with POLS_JAEV5 structural polypeptide, POLS_JAEVM structural polypeptide and POLG_JAEVM (genome polypeptide *Japanese encephalitis virus (strain M28) (JEV)*) respectively. 6 ligand i.e. Prechlorperazine, Chlorpromazine Hydrochloride, Chlorpromazine Methiodide, Methochlorpromazine, Chloracyzine and N-Dimethylmethanimidamide were selected to perform the docked again protein based on literature studies. Docking was carried out using the Schrodinger application software using Glide Dock methods. The docking result of all three proteins against ligand suggests that Chlorpromazine Hydrochloride is the best inhibitor indicating a docking score of range from -4.954. This study identified a potential ligand against JEV and can be further used for drug discovery and development.

Key Words: Japanese Encephalitis Virus, Japanese encephalitis, Docking, Ligand detection, Glide dock

INTRODUCTION

JE is an inflammatory disease that is caused by JEV. The virus includes a single complementary strand of RNA virus. This virus belongs to the family of flavivirus (Flaviviridae). JEV virus is almost similar to the West Nile encephalitis virus. JE virus-induced inflammation contributes to disease severity by inducing neuronal cell death¹inhibiting the proliferation and differentiation of neural progenitors and disrupting the blood-brain barrier (BBB). Japanese encephalitis (JE) is very often a viral disease of viral encephalitis in living organisms including human and animals both particularly in Southeast Asia and also show some smaller extent in the Western Pacific regions and Australia.JEV proceeds to occupy additional geographic locations and producing a significant impact on general health issues.²

Japanese encephalitis (JE) is an infected brain cell that can affect by the Japanese encephalitis virus (JEV). The vi-

rus is transmitted by the mosquito and vectors of another organism, so it is also known as the mosquito-borne disease of the Japanese encephalitis virus (JEV). JEV contains a positive-sense ssRNA of both approx 11 kb, the virus can contain several structural proteins include (C, M and E) and 2 non-structural proteins include (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).³

The crucial role of E protein includes illness against pathogen importance in immunogenicity membrane fusion virus and virus maturation. JE is a disease and organisms sponsor such as creatures and cows that play an important function inside magnification and the conserved of their virus whereas ancestral bunch, mosquitoes had been subjected to the transportation of virus.⁴

The JEV is transmitted from one living organism to another organism through the mosquitoes that were first discovered in the 1930s. In 1938 it was found in *Culex tritaeniorhynchus*.

Corresponding Author:

Ruchi Yadav, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India.
Email: ryadav@lko.amity.edu

ISSN: 2231-2196 (Print) ISSN: 0975-5241 (Online)

Received: 12.08.2020

Revised: 22.10.2020

Accepted: 18.12.2020

Published: 05.07.2021

It is mostly found in Southern Asia, Eastern Asia and *C. tritaeniorhynchus* is the main vector for responsible JE infection and context in Northern Australia, *C. Annulirostris* is the vector that caused the infection.⁵

However, the JE virus was characterized by different sort of species of mosquitoes and also is still a member of genus *Culex*, *Anopheles* and *Mansonia* however sophistication of *Culexvishnui* which contain the *Culex tritaeniorhynchus*, *Culexvishnui* and also *Culexpseudovishnui*) that are found in the subordinate of vectors of JEV that could be breed in living human body of still water because paddy fields.⁶

The virus can be shown in human and animals basically in cattle, horses and are degrade-end hosts of JEV like they are unable to producing enough level of viremia to reintroduce the infection to other mosquitoes⁷. Transmission of JE in human beings is not feasible, even though transfusion-related JEV transmission was described in two immune balanced lung transplant receiver in Hong Kong and the result of lung transplant in the case of one several encephalitis and other case is asymptomatic inflammation with seroconversion.⁸

Many different secondary vectors are shown from other studies for example *Mansoniaindiana*, *C. whitmorei*, *C. gelidus*, *C. epidesmus*, *Anopheles subpictus*, *A. peditaeniatatus*, along with *M. uniform*. The pure cycle of the JE virus calls for water creatures and *Culex* mosquitoes although dinosaurs are deemed to function as absolutely the absolute most crucial amplifying host, supplying a link to individuals throughout their proximity into housing. In India, the most vector is *Culextritaeniorhynchus* members of *C.vishnui* and *C. pseudovishnui*. However secondary vectors like *C.gelidus*, *C.fuscocephala*, *C. whitmorei*, *Anopheles subpictus* and *M. uniform* are likewise accountable for transmission of JE in India.⁹

The cycle of the JE virus involves *Culex* and aquatic birds while pigs are regarded to function as the amplifying host, providing a link to folks throughout their closeness to home.¹⁰ Individuals are believed to become dead-end hosts. JEV is significantly affecting our developing country India and has been emerged as a potential hub of JEV infection.¹¹

Despite some recent successes with widespread vaccination, JE will likely remain an important public health problem and significantly affecting our country India. From the path mechanism study of JE viral infection disease inflammation is the key process in regulation CNS microenvironment and activation of microglia cells takes place followed by JEV infection and associated with progressive neurodegeneration.¹²

Japanese encephalitis (JE) is among the most frequent cause of viral encephalitis in humans and can be found worldwide, notably in south-east Asia and less commonly in the Western Pacific regions and Australia. JEV sustained generating a medical-related difficulty and to occupy precisely the unique places.¹³This virus can be infected the brain cells during stimulation of microglial cells which is brought on by the JE virus even the mosquito-borne Japanese encephalitis virus (JEV) belongs to the genus *Flavivirus* of the family *Flaviviridae*.¹⁴

JEV comprises a stranded ssRNA containing approximately 11 kb, where E, M and C encoded by NS1, NS2A, NS2B, NS3, NS4A, NS3, NS4B, NS5 assessed by the 7 protein and the 3 structural components¹⁵. The E protein performs an important part in immunogenicity, virus-cell membrane fusion, infection, and virus maturation. Detail of JEV protein is shown in **table 1**. JE is primarily a zoonotic illness and vertebrate hosts such as pigs and birds play a significant role within the maintenance and amplification of the virus whereas invertebrate host, mosquitoes are responsible for the transmission of the virus.¹⁶

Table 1: Detail of Genome and Structural Polyprotein of JEV

S. No.	Uniport Id	Entry Name	Protein Name	Organism
1	P27395	POLG_JAEV1	Genome polyprotein	Strain (SA-14)
2	P32886	POLG_JAEVJ	Genome polyprotein	Strain (Jaoars982)
3	PoDOH8	POLS_JAEVJ	Structural polyprotein	Strain (Jaoars982)
4	G3FEX6	POLG_JAEVM	Genome polyprotein	Strain (M28)
5	P19110	POLG_JAEV5	Genome polyprotein	Strain (SA(v))
6	P14403	POLS_JAEVN	Genome polyprotein	Strain (Nakayama)
7	PoDOH7	POLS_JAEV1	Structural polyprotein	Strain (SA-14)
8	PoDOH9	POLS_JAEV5	Structural polyprotein	Strain (SA(v))
9	PoDOK8	POLS_JAEVM	Structural polyprotein	Strain (M28)

Functional Study of JEV Protein

Capsid protein C plays an important role in virus potential along the binding to the cell membrane including also the viral RNA through a nucleocapsid further inducing the mature virus molecules at the time of entry, the capability of introducing genome into the cytoplasm on suffocating the host till hemifusion induced by the surface of the protein. It can move to the cell nucleus by cytoplasm and perform functions.¹⁷ It can migrate from the cytoplasm to the cell nucleus where it performed host functions. Host EXOC1 is controlling through the antiviral effects by disassemble and deactivate the closed end through the proteasome degeneration pathway. The capsid protein C prevents the RNA silencing along with mess with host Dicer and the Peptide inhibits the ultimate fusion act of envelope proteins in trans-Golgi by required to bind to envelope protein E and maintain the standard pH6.0.¹⁸

After that virion delivers in extracellular space, gets disrupted from E dimers. The protein envelope attaches to the host cell surface receptor and interferes with the fusion along with the viral and cellular membrane.¹⁹ Envelope protein which is a heterodimer alongside protein prM is incorporated in the endoplasmic reticulum. The virion growing assumes a significant job in the ER and recently combined juvenile particles is covered with 60 spikes which comprise a heterodimer encompassed by forerunner pr M and envelope protein E. the virion is moved to the Golgi mechanical assembly where less pH cause partition of prM-E heterodimers and age of E same dimers.²⁰

Homology Modeling

The Major attention of structural biology included the creation of protein-ligand Complexes in which the protein molecules employ vigorous in the course of binding. Consequently, perceptive of protein-ligand interaction will be very

important for structure-based drug design and its direction. The forecast of the 3D arrangement of a Protein from the amino acid chain remains a basic logical and scientific issue.

This problem approach can often have achieved using different types of Techniques and the first and most accurate approach is “homology” modelling or “relative. Homology Simulating is simply the approach of the decision to generate a dependable protein version of 3 D from its chain of amino acids. The Idea of homology Simulating is employed to search the exact conformation space by minimally disturbing.

Molecular Docking

In the study of atomic demonstrating, docking is utilized as a technique that can give results or predicts the direction inclination of one particle to a second when they are bound to like each other strategy and structure a steady mind-boggling. To anticipate the quality of affiliation or restricting liking between two particles, the information on this favoured direction approach thusly might be utilized. Sub-atomic docking is one of the most now and again utilized techniques in tranquillize structure-based plan, given its capacity to distinguish and foresee the coupling compliance of little particle ligands to the objective restricting site of assignment. The coupling conduct of Characterization has a significant job in the plan of medications just as to key biochemical procedures.²¹

MATERIALS AND METHOD

JEV proteins were searched in the UniProt database table 2 shows the list of JEV protein. These proteins were selected as protein target for docking study and screening of best ligand.

Table 2: Identified target protein of jev with UniProt id

S. No.	UniProt Id	Entry Name	Protein Name	Organism
1	PoDOH9	POLS_JAEV5	Structural polyprotein	Strain (SA(v))
2	PoDOK8	POLS_JAEVM	Structural polyprotein	Strain (M28)
3	G3FEX6	POLG_JAEVM	Genome polyprotein	Strain (M28)

Table 3 shows the list of ligands that were selected for docking with the target protein of JEV. These ligands were retrieved from the PubChem database against the Structural and genome polyprotein of JEV.

Table 3: Identified ligand with PubChem CID

S. No.	PubChem CID	Molecular Formula	Molecular Weight	Ligand Name
1	4917	C ₂₀ H ₂₄ ClN ₃ S	373.9 g/mol	Prechlorperazine
2	6240	C ₁₇ H ₂₀ Cl ₂ N ₂ S	355.3 g/mol	Chlorpromazine Hydrochloride
3	9682	C ₁₈ H ₂₂ ClN ₂ S	460.8 g/mol	Chlorpromazine Methiodide
4	9683	C ₁₈ H ₂₂ ClN ₂ S ⁺	333.9 g/mol	Methochlorpromazine
5	13113	C ₁₉ H ₂₁ ClN ₂ OS	360.9 g/mol	Chloracyzine
6	58273	C ₁₈ H ₂₀ ClN ₃ S	345.9 g/mol	N- Dimethylmethanimidamide

RESULTS AND DISCUSSION

I. Homology Modeling of POLS_JAEV5 Protein (UniProt Id: P0DOH9)

The structure prediction of structural Polypeptide was done by using automated modelling SWISS-MODEL (<http://swissmodel.expasy.org>) and the modelled protein structure was verified using Ramachandran plot as shown in figure 1a, which shows the protein structure and 1b show the Ramachandran plot.

Figure 1: Result of JEV showing the modelled structure of structural polypeptide (UniProt ID- P0DOH9) (a) and Ramachandran plot for protein STRUCTURAL ANALYSIS (b)

Ramachandran plot determines the analysis of amino acids residues and detailed analysis shows that 90.9% of the residue lies in most favoured regions, 7.2% residues in allowed regions and 0.9% residues in the disallowed region as shown in fig 1(b).

Ligands were identified from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>), and docking was done using Schrodinger maestro. POLS_JAEV5 Protein shows the best docking with N-Dimethylmethanimidamide ligand with a docking score of - 3.85. The docking result with all the ligands was shown in table 4.

Interaction map between ligand and the target protein as shown as mentioned in table 4 and interaction graphs between protein and ligands were shown in figure 2A-F

Figure 2: Result of Schrödinger Maestro showing the Ligand interaction map with structural polypeptide (POLS_JAEV5). Each interaction map (A-F) represents the type of bond and interacting amino acids where -> (Hydrogen bond backbone),> (Hydrogen bond side chain) and straight-line represents pi-pi interaction.

Table 4: Docking score of the interaction of ligands with target POLS_JAEV5 protein

S. No.	PubChem CID (Compound identification number)	Ligand Name	Docking Score	Ligand-Protein interaction map
1	58273	N- Dimethylmethanimidamide	-3.855	A
2	13113	Chloracyzine	-3.544	B
3	9682	Chlorpromazine Methiodide	-3.384	C
4	9683	Methochlorpromazine	-3.384	D
5	6240	Chlorpromazine Hydrochloride	-3.375	E
6	4917	Prechlorperazine	-3.36	F

II. Homology Modeling of POLS_JAEVM Protein

The structure prediction of structural Polypeptide was done by using automated modelling SWISS-MODEL (<http://swissmodel.expasy.org>) and protein structure verification was done using Ramachandran plot analysis. The result of structure prediction and verification is shown in figure 3a and 3b respectively.

Figure 3: Result of JEV showing the modelled structure of structural polypeptide (UniProt ID- P0DOK8) (a) and Ramachandran plot of modelled protein (b)

Ramachandran plot analysis of modelled POLS_JAEVM Protein shows that 88.1% of residues lie in most favoured regions, 10.1% residues in allowed regions and 0.7% residues in the disallowed region as shown in figure 3(b).

Docking result of POLS_JAEVM Protein with ligands as shown in table 5, shows that POLS_JAEVM Protein has best interaction with Chlorpromazine Hydrochloride ligand with the docking score of - 3.88 as shown in table 5.

Table 5: Docking score of the interaction of ligands with target POLS_JAEVM protein. The docking score shows the strength and stability of interaction, and the interaction Map of each compound was shown in corresponding figures as mentioned in the table.

S. NO.	PubChem CID	Ligand Name	Docking Score	Interaction Map
1	6240	Chlorpromazine Hydrochloride	-3.88	A
2	58273	N- Dimethylmethanimidamide	-3.859	B

Table 5: (Continued)

S. NO.	PubChem CID	Ligand Name	Docking Score	Interaction Map
3	4917	Prechlorperazine	-3.471	C
4	9682	Chlorpromazine Methiodide	-3.441	D
5	9683	Methochlorpromazine	-3.441	E
6	13113	Chloracyzine	-3.331	F

The interaction map of POLS_JAEVM Protein with ligands as mentioned in table 5 was shown in figure 4A-F.

Figure 4: Result of Schrödinger Maestro showing the Ligand interaction with structural polypeptide (POLS_JAEVM).

III. Homology Modeling of POLG_JAEVM Protein

The structure of POLG_JAEVM Protein is shown in figure 5a and the Ramachandran plot in figure 5b. Ramachandran plot of the modelled protein shows that 90.4% lies in most favoured regions, 9.3% residues in allowed regions and 0.1% residues in the disallowed region.

Figure 5: Result of JEV showing the modelled structure of GENOME POLYPROTEIN (UniProt ID- G3FEX6).

The molecular docking study of POLG_JAEVM shows that it has the best interaction with Chlorpromazine Hydrochloride ligand with a docking score of -4.954 as shown in table 6.

Table 6: Docking score of the interaction of ligands with target POLG_JAEVM Protein

S. NO.	PubChem CID	LIGAND NAME	DOCKING SCORE	(FIG: 4.3.2(B))
1	6240	Chlorpromazine Hydrochloride	-4.954	A
2	58273	N- Dimethyl-methanimidamide	-4.836	B
3	13113	Chloracyzine	-4.559	C
4	9682	Chlorpromazine Methiodide	-4.122	D
5	9683	Methochlorpromazine	-4.122	E
6	4917	Prechlorperazine	-4.04	F

The interaction map between ligands and POLG_JAEVM as mentioned in table 6 was shown in figure 6e-f.

Figure 6: Result of Schrödinger Maestro showing the Ligand interaction with genome polyprotein (POLG_JAEVM).

CONCLUSION

JEV proteins were studied and results were obtained from UniProtKB. Uniprot ID for 7 different structure and genome Polyprotein of Japanese encephalitis was retrieved and studied. Variants of structural and genome Polyprotein from different JEV strain were also studied and their structure was modelled using the SWISS MODEL software tool. Further Schrödinger. the software was used for docking with the identified ligands and a protein-ligand interaction map was studied. According to different researches in JEV, it has been identified that a different strain of JEV protein causes virus-induced encephalitis development in *mosquito-borne flavivirus*²². Ligand which can play as the antagonist for this protein can be regarded as a drug against inflammation disease.

The First Docking result shows that POLS_JAEV5 (Uniprot id: **P0DOH9**) protein have the best interaction with N- Dimethylmethanimidamide ligand with a docking score of -3.85, second Docking result shows that POLS_JAEVM (Uniprot id: **P0DOK8**) protein have the best interaction with Chlorpromazine Hydrochloride ligand with docking score of -3.88 and the third Docking result shows that POLG_JAEVM (Uniprot id: **G3FEX6**) protein have the best interaction with Chlorpromazine Hydrochloride ligand with docking score of -4.954. Among these 3 interactions of ligands against a target protein, the best interaction shows that the genome Polyprotein POLG_JAEVM (Uniprot id: **G3FEX6**) with Chlorpromazine Hydrochloride ligand with the highest docking score of -4.954. It could be a possible compound for the treatment for targeting the JEV pathway.

ACKNOWLEDGEMENT

The authors acknowledge the immense help received from the online database and bioinformatics tools. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Conflict of Interest

There is no Conflict of Interest.

Source of Funding

No funding was provided for this research.

REFERENCES

- Hills SL, Walter EB, Atmar RL, Fischer M, Group AJ, Group AJ, Walter Jr E, Atmar RL, Barnett E, Barrett A, Bocchini JA. Japanese encephalitis vaccine: recommendations of the advisory committee on immunization practices. *MMWR Recommend Rep*. 2019 Jul 19;68(2):1.
- Pearce JC, Learoyd TP, Langendorf BJ, Logan JG. Japanese encephalitis: the vectors, ecology and potential for expansion. *J Trav Med*. 2018 May;25(Suppl_1): S16-26.
- Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. *Hum Vacc Immunoth*. 2017 Jun 3;13(6):1320-37.
- Wang X, Li SH, Zhu L, Nian QG, Yuan S, Gao Q, Hu Z, Ye Q, Li XF, Xie DY, Shaw N. Near-atomic structure of Japanese encephalitis virus reveals critical determinants of virulence and stability. *Nat Commun*. 2017 Apr 26;8(1):1-9.
- Oliveira AR, Cohnstaedt LW, Strathe E, Etcheverry L, McVey DS, Piaggio J, Cernicchiaro N. Meta-analyses of Japanese encephalitis virus infection, dissemination, and transmission rates in vectors. *Am J Trop Med Hyg*. 2018 Mar 7;98(3):883-90.
- Velsankar K, Sudhakar S, Maheshwaran G. Effect of biosynthesis of ZnO nanoparticles via Cucurbita seed extract on *Culex tritaeniorhynchus* mosquito larvae with its biological applications. *J Photochem Photobiol B: Bio*. 2019 Nov 1;200:111650.
- Ricklin ME, García-Nicolás O, Brechbühl D, Python S, Zumkehr B, Nougaière A, Charrel RN, Posthaus H, Oevermann A, Summerfield A. Vector-free transmission and persistence of Japanese encephalitis virus in pigs. *Nature Commun*. 2016 Feb 23;7(1):1-9.
- Chai C, Palinski R, Xu Y, Wang Q, Cao S, Geng Y, Zhao Q, Wen Y, Huang X, Yan Q, Ma X. Aerosol and Contact Transmission Following Intranasal Infection of Mice with Japanese Encephalitis Virus. *Viruses*. 2019 Jan;11(1):87.
- Pearce JC, Learoyd TP, Langendorf BJ, Logan JG. Japanese encephalitis: the vectors, ecology and potential for expansion. *J Trav Med*. 2018 May;25(Suppl_1): S16-26.
- Yu X, Zhu Y, Xiao X, Wang P, Cheng G. Progress towards understanding the mosquito-borne virus life cycle. *Trend Parasit*. 2019 Dec 1;35(12):1009-17.
- Sun P, Nie K, Zhu Y, Liu Y, Wu P, Liu Z, Du S, Fan H, Chen CH, Zhang R, Wang P. A mosquito salivary protein promotes flavivirus transmission by activation of autophagy. *Nature Commun*. 2020 Jan 14;11(1):1-5.
- Karthikeyan A, Shanmuganathan S, Pavulraj S, Prabakar G, Pavithra S, Porteen K, Elaiyaraja G, Malik YS. Japanese encephalitis, recent perspectives on virus genome, transmission, epidemiology, diagnosis and prophylactic interventions. *J Expt Bio Agri Sci*. 2017 Dec 1;5(6):730-48.
- De Wispelaere M, Desprès P, Choumet V. European *Aedes albopictus* and *Culex pipiens* are competent vectors for Japanese encephalitis virus. *PLoS Trop Dis*. 2017 Jan 13;11(1):e0005294.
- Ruget AS, Beck C, Gabassi A, Trévenec K, Lecollinet S, Chevalier V, Cappelle J. Japanese encephalitis circulation pattern in swine of northern Vietnam and consequences for swine's vaccination recommendations. *Transb Emerg Dis*. 2018 Dec;65(6):1485-92.
- Zheng X, Tong W, Liu F, Liang C, Gao F, Li G, Tong G, Zheng H. Genetic instability of Japanese encephalitis virus cDNA clones propagated in *Escherichia Coli*. *Virus Gen*. 2016 Apr 1;52(2):195-203.
- Han SR, Lee SW. Inhibition of Japanese encephalitis virus (JEV) replication by specific RNA aptamer against JEV methyltransferase. *Biochem Biophys Res Commun*. 2017 Jan 29;483(1):687-93.
- Poonsiri T, Wright GS, Solomon T, Antonyuk SV. Crystal Structure of the Japanese Encephalitis Virus Capsid Protein. *Viruses*. 2019 Jul;11(7):623.
- Bhardwaj T, Saumya KU, Kumar P, Sharma N, Gadhave K, Uversky VN, Giri R. Japanese Encephalitis Virus: Exploring the dark proteome and disorder-function paradigm. *The FEBS J*. 2020 May 30.
- Tangkananond W, Lomonosoff G. The Expression of Japanese Encephalitis Virus Envelope Gene in Green Cos. *Access Micro*. 2019 Dec 1;1(10):100.
- Yang J, Yang H, Li Z, Wang W, Lin H, Liu L, Ni Q, Liu X, Zeng X, Wu Y, Li Y. Envelope protein mutations L107F and E138K are important for neurovirulence attenuation for Japanese encephalitis virus SA14-14-2 strain. *Viruses*. 2017 Jan;9(1):20.
- Roy U. Structural and molecular analyses of functional epitopes and escape mutants in Japanese encephalitis virus envelope protein domain III. *Immunol Res*. 2020 May 22:1.
- Bajaj S, Garg D, Agrawal N, Mandowara SL. Value of rapid antigen assay in the diagnosis of pyogenic and partially treated meningitis. *Int J Curr Res Rev*. 2013 Aug 15;5(16):47.