“MUTATIONAL ANALYSIS OF MERS CoV VARIANTS, LESSONS TO PREVENT FUTURE PANDEMICS”

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT OF THE DEGREE
OF
MASTER OF TECHNOLOGY
IN
INDUSTRIAL BIOTECHNOLOGY

Submitted by
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CERTIFICATE

I hereby certify that the project dissertation titled **'Mutational Analysis of MERS-CoV variants, lessons to prevent future pandemics'** which is submitted by **Ayushi Pandey**, **2K20/IBT/03**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology,is a record of the project work carried out by the student under my supervision. To the best of myknowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

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CANDIDATE'S DECLARATION

I, Ayushi Pandey, 2K20/IBT/03, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled **'Mutational Analysis of MERS CoV variants, lessons to prevent future pandemics'** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied fromany source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.

Place: Delhi

Date: 26-05-2022

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ABSTRACT

Initially, MERS-CoV was isolated in 2012 in Saudi Arabia, where it caused epidemics and major health emergencies, with mortality rates as high as 40%. However, due to the containment of the epidemic, mainly in Saudi Arabia and other Middle Eastern countries, it did not take the shape of a pandemic, unlike the case of the recent SARS-Cov-2 outbreak. Protein-protein docking has confirmed that DPP4 is the host cell receptor where MERS-CoV binds with the help of its structural spike protein. In the present study, we have studied different MERS-CoV variants to compute the comparative assimilation of the mutational hotspots and their binding efficiencies with the human receptor. The M1099R and L449F Orf 1a/b spike protein variants were found to bind with the highest binding energy to the receptor DPP4 and these mutations were also reported in the most virulent variants reported till date. We have further identified the stretch of peptide residues that may serve as the putative mutation hotspot of MERS-CoV that may lead to future pandemics and the more conserved regions that may serve as potential vaccine candidates with greater global coverage.

KEY WORDS: MERS-CoV; Virulent mutation; vaccine candidate; Conserved Region; Protein-protein docking.

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CHAPTER 1- INTRODUCTION

In June 2012, Saudi region experienced the first cae of the Middle East respiratory syndrome (MERS), that occurred due to variant of betacoronavirus. It spread to 27 countries, causing 2,519 infections and 866 deaths and a CFR of 34.4%, by the end of January 2020 [1]. Experts studied that the MERS-CoV originated in bats, later spreading from infected dromedary camels to humans [2]. The spike (S) protein of B-CoV plays important role in cross-species transmission by permitting virus-receptor recognition and triggering viral pathogenesis. The receptor-binding domain (RBD) on the S protein's N-terminal is necessary for the viral penetration through the host cells. Further, the acuteness of cross-species efficiency along with infections is known by the mutations in CoV RBD. The infectivity of MERS-CoV is reliant on the 5-day median incubation period for man-to-man transmission, varying from 2 to 14 days. Roughly, the incubation period of MERS-CoV illness to hospitalization is 4 days, with a highest duration of 5 days for severely ill patients admitted to the intensive care unit (ICU), and about 12 days from the appearance of viral symptoms to mortality in more than 30% cases [3]. Various transmission inspection has revealed that MERS-CoV has a debase reproduction number (R_0) than SARS-CoV (0.69 vs 0.80) and has not achieved pandemic potential [4]. Immunosuppression due to malnutrition, stress, deficiencies, concurrent infections, and many more, could exasperate the MERS-CoV infection course.

The genetic data of MERS-CoV shows presence of 30,119 nucleotides along with 10 presumed open reading frames [5] [6]. The 5'end region of the genome consists of orf 1a and orf 1b, while the 3'end ciphers the main proteins like a spike (S), nucleocapsid (N), membrane (M), and envelope (E), together with several accessory proteins such as 4a, 3,4b, 8b, and 5 [7] [8]. The mutation in the RBD domain on the N-terminus of S-proteins has the greatest influence on cross-species transmission and virus severity [9][10] [11]. Significant positive selection has been found

on 9 sides of the protein, indicating that the S-protein has been subjected to intense evolutionary pressure throughout its cross-species transmission [12] [13].

The present study focuses on the major amino acid hotspots within the S-protein and Orf 1a/b as they play a crucial role in host penetration during transmission. The knowledge of the mutation hotspots within the genome can control further pandemics by using it to predict potential vaccine candidates with redundancies that would cover multiple variants and also identify conserved stretches that may be crucial for wide vaccine coverage. Further, our study of the identification of mutational hotspots and their correlation with disease severity and transmissibility will help design effective treatments against the virus even for such strains that are likely to evolve.

CHAPTER 2- REVIEW OF LITERATURE

2.1 Virus

The name virus has its origin from a Latin word contexting “poison”. These are small-sized infectious agents having an uncomplicated composition having capacity to only multiply in plants, animals, or bacterial living cells. In 1898 Dutch scientist Martinus W. Beijerinck gave the term contagium vivum fluidum to viruses indicating that they are reproducing, live organisms different from the present ones. He later along with another Russian scientist Dmitry I. Ivanovsky concluded that these are the agents responsible for the transmission of disease in tobacco plants, later known as tobacco mosaic virus. The unique features of viruses led to new methods to study and classify them. The British scientists Christopher Andrewes, Patric P. Laidlaw, along with Wilson Smith successfully transferred influenza to ferrets which were later adapted to mice, in 1933. Another American scientist George Hirst investigated the influenza present in the tissue of the embryo of the chicken can be suspected by the ability of agglutination of red blood cells. In 1949, an important study was revealed by American scientists Frederick, John Enders and Thomas Weller by developing cell culture techniques. These cells could then be infected with viruses responsible for different diseases. This allowed development and manufacturing of vaccines against discrete viral diseases. Coming of the electron microscope in the 1940s, allowed the individual particles of the virus to be easily seen easily, enabling the viral classification and giving information about its structure.

Development in the field of physics, molecular biology, and chemistry, following the 1960s has transformed the study of viruses. For instance, electrophoresis offered a better perception of the viral protein and its nucleic acid content. More-advanced immunologic writ, like the usage of monoclonal antibodies pointed to certain antigenic sites, gave a better vision of the viral proteins.

The shift in molecular biology area enabled the study of genetic particulars ciphered in nucleic acids responsible for reproduction, synthesizing distinctive proteins, and altering cellular role. With the finding of giant aquatic viruses in the early 21st century, the ecological importance of these viruses in the environment was realized.

2.2 General Features

2.2.1 Definition: Viruses are special as they don't belong to any pertaining kingdom (plants, animals, or bacteria), rather they have a kingdom of their own. In fact, these viruses aren't obliged to even be called organisms because they are dependent on their host cell for reproduction and other metabolic processes.

The viruses either having DNA or RNA, accompanied by protein are called true viruses. The nucleic acid is responsible for having the genetic information distinctive for each virus. The infective devise of the virus exterior to its host cell is known as the virion, containing minimum one especial protein made by genes existing in the viral nucleic acid. These synthesized proteins incarnate a shell throughout the nucleic acid known as capsid. Few viruses have proteins inside their capsid; adjutant as enzymes, during nucleic acid union. Viroid is organisms comprising nucleic acid but dearth structural proteins. Viruses cannot synthesize proteins of their own due to absence of ribosomes responsible for translation. These are power parasites as they cannot form energy as ATP, rather they derive energy from the host cell. The attacking virus utilizes the amino acids of the proprietor cells to construct its own proteins. The major contagious bit of any virus is the nucleic acid (either DNA or RNA).

The capsid has three functionalities:

- to save its nucleic acid from digesting enzymes,
- to enable the attachment of virion to the receptors present on the host cell,
- to furnish proteins responsible for the virion penetration through the surface membrane.

2.2.2 Host range and distribution: In most of the cases the host radius and dissemination of viruses are one of the factors responsible for their classification. Traditionally viruses are divided into three class:

- that infect animals
- that infect plants
- that infect bacteria

Usually, the plant viruses are hand on via insects that cater on plants. The provider of animal viruses ranges from simple organisms to humans. Some animal viruses affect either invertebrate animals or vertebrates, while some affect both. The arthropods are responsible for causing serious human diseases. Some viruses are restrained in their host range to the several form of vertebrates. Certain viruses grow only in ectothermic vertebrates reproducing at low temperature

2.2.3 Size and Shape: The number and arrangements of proteins along with nucleic acid decides their size and shape. The proteins along with nucleic acid of each class of viruses gather to form nucleoprotein or Nucleo-capsid. Certain viruses have several protein layers around the nucleic acid; while, some have a lipoprotein membrane surrounding the core of the nucleocapsid. Deep down the membranes are extra proteins that decide the virus-host cell specificity. The nucleoprotein has unique properties for each cell of the virus responsible for resolving the size and

shape. The genome of the largest known viruses namely Mimiviruses and Pandoraviruses usually ranges from 1-2.5 Mb.

2.3 The Cycle of Infection

Viruses depends on the host cell for reproduction. The virion produces countless progeny, generally similar to the parent virion. The viral activity mainly depends on its ruinous capacity against a set host cell and on surrounding conditions. The multiplication of the brood is quick during the vegetative tenure of viral septicaemia. This process usually led to cell death releasing various virus progeny. Some viruses are called latent as the multiplication does not lead to rapid cell death. Cells containing temperate viruses are known as lysogenic as the cells break down when they run into physicochemical, like ultraviolet light. Additionally, different viruses, the genetic information not unified in host genome, may be dormant in tissues for lengthy duration besides leading to tissue vandalization.

Although the reproductive pathways of various viruses differ, there are specific cycle of infection in viruses. The cycle starts with the infection of virion by attaching to the host cell facet (adsorption).Next, the penetration of virion on the outer membrane to the interior of cell (cytoplasm) or injection of the genetic material of the virus into the cell interior while the protein capsid is on the cell surface. During the whole-virion penetration, a successive process (uncoating) releases the genetic material from the protein capsid and envelope.

Plant cells have firm cell walls, which plant viruses cannot easily penetrate. Plant viruses have not progressed in developing their own systems for infecting the host cells, and so they are transferred via the insects that feed on plants. During experiment, Plant virus can infect plant cell only if its protoplast is devoid of cell or its cell walls are rubbed off using sandpaper.

Viral infection of animal cells involves various processes, as the animal cells are not enclosed through walls but via flexible membrane of lipoprotein bilayer. Several animal viruses, penetrate cells through process known as endocytosis.

2.4 MERS-CoV Virus

The MERS is a single-stranded coronavirus from genus beta coronavirus and family coronaviridae. Transmission mechanism is not well designated. The MERS virus is genetically alike to coronaviruses of bat and majorly detected in African and the peninsular animals mainly camels. The exposure to such camels is danger for MERS, but not much information is known about specific exposure leading to human cases. Cases suggest that the virus mainly spread by close contact leading to family and later community transfer. However, continuous community passing of MERS has not been seen.

MERS coronavirus leads to serious respiratory illness, and nearly 35% of confirmed cases have been lethal. Risk persists in Arabian region. The cases mentioned have travelled to Middle East countries recently. MERS cases has also been notified in passengers returning to countries like America, Europe, Asia, and Africa from mentioned regions.

Various detecting assays have been made to encounter infection of MERS coronavirus, like real-time RT-PCR. Bottom respiratory samples including sputum, bronchoalveolar lavage are the main testing specimens, although the upper respiratory specimens should also be considered. To enhance the detection process of the virus, several specimens from mentioned sites should be collected during the illness.

In addition to this, the health care officials should isolate MERS patients having fever and related symptoms within 14 days from traveling from Arabian regions or have had near contact with latest symptomatic traveler from this region.

Particular antiviral is not available against MERS. The treatment is restricted to supportive care. Suspected MERS patients are advised airborne infection control standard precautions. Vaccines or preventive drugs are under process against MERS virus. The travelers should maintain precautionary protocol while travelling to avoid any further transmission, including frequent handwash, ignore touching eyes, nose and mouth and avert direct contact with infected person. Individual with prior medical history like diabetes, lung disease, kidney failure are prone of getting the infection.

2.5 Pathogenesis of MERS-CoV

The MERS attaches to dipeptidyl-peptidase 4 (DPP4) to infect human cells. After attachment to the facet of the host cell, the virus enters the cytoplasm of the host cells by using proteases like TMPRRS2 and cathepsin [14]. These proteases then carry out the cleft of the S-protein, after which the blend of the viral and host cell membranes takes place. This S protein segregation takes place at two distinct positions in its the S2 domain. The initial cleavage leads to the separation of the RBD and fusion domains, followed by the cleavage that tends to expose the fusion peptide. This whole event mostly occurs in the endosomes [15]. The option of MERS-CoV to employ some host proteases is affected by the target cell and the cleavage stage of their S protein before contamination [16] [17].

Cellular proteases gather in different subcellular locations on the entry pathway of endocytic CoV: serine proteases like elastase and plasmin are extracellular; the type II transmembrane serine proteases (TTSPs) are embedded into the plasma membrane, and the endosomes are enriched with cysteine-type cathepsin proteases [18]. In major CoV infections, not all of these proteases are needed [19]. However, each has a different potential to activate the viral fusion as a result of

productive infection. Knowing these preferred routes and their link to virus-induced disease enables one to spot viral variants that might have high transmissibility and to identify the host factors that might be targeted therapeutically so that infections are repressed at an early stage [20] [21]. Apart from bats, humans, and camels, other animal species, including horses, pigs, goats, sheep, rabbits, etc., have also been suggested as possible hosts of the virus by identifying the S-protein of MERS CoV [22] [23].

Effective vaccines are crucial for averting MERS-CoV infection and consequent pandemics [24]. Various S protein-based vaccines are under process, which show efficacy against MERS-CoV when tested on animal models [25] [26]. Different agents, like those that hinder viral replication, block the entry of the virus, or impede the immune system of the host, have been tried [27] [28]. Seeing the prior studies with the SARS CoV virus, the use of monoclonal antibodies, hyperimmune globulin, or plasma therapy may be effective and is considered as first-line treatment [29] [30]. In vitro studies and animal experiments, revealed that ribavirin and interferon alpha-2b, when used together, can produce promising results [31] [32]. The various suggested agents were screened for their potential therapeutic efficacy by Dyall et al. [33] [34].

CHAPTER 3- METHODOLOGY

3.1 Sequence and data Retrieval

The sequence of S-protein and Orf 1a/b was fetched from NCBI with the accession numbers QBF80510.1 and QKF93417.1 respectively. The DPP4 host cell receptor was obtained from the NCBI under the accession number AAH13329.

3.2 Mutation

The hotspot mutation was studied by introducing a mutation in the downloaded PDB structure of S-protein and Orf 1a/b according to their hot spot amino acid mutation and saved in PDB format.

3.3 Swiss Model

The 3-D structure of MERS CoV was obtained in PDB format on the basis ofa high similarity percentage of the sequence matched to the reference sequence.

3.4 HEX 8.0

This tool was used to obtain the protein-protein docking results of structural proteins (S-protein and Orf 1a/b) of MERS CoV and DPP4.The end result gave us the E-total between ligand (MERS CoV) and receptor (DPP4) along with the number of H-bonds involved in their binding.

3.5 HDOCK

HDOCK is a template-based, powerful pipeline for protein-protein docking. It differs from other docking platforms as it assists amino acid sequences as input while adjusting the ligand binding. The template sequences of the receptor and the ligand were submitted for obtaining the binding

energy of amino acid residue, docking score, and RMSD (in Armstrong). HDOCK gives the top 20 docked models of the provided template, out of which the model with the lowest RMSD value was chosen, as the lowest RMSD value provides a perfect docking structure. The 3-D docked structure was saved in PDB format.

3.6 PyMOL 2.4

The overall result of HDOCK was examined with PyMOL for a better understanding of the binding between the receptor and the ligand.

CHAPTER 4- RESULT AND DISCUSSION

The whole-genome order of the MERS virus was downloaded using NCBI (GCF_000901155.1). Spike glycoprotein and the Orf 1a/b FASTA sequence were accessed via accession numbers QBF80510.1 and QKF93417.1, respectively. The FASTA sequence was pasted on the SWISS model, and the protein structures of spike and Orf 1a/b were downloaded with PDB IDs 7krs.1 and 6jx7.1, respectively.

Hotspot amino acid analysis of spike and Orf 1a/b was obtained through various literature reports [35] [36] [37] [38] [39] [40]. Epidemiological evidences suggest that the major mutations took place in the orf 1a/b and S-protein within the MERS CoV genome from the onset of infection till date. Therefore, we focused our studies on these regions, narrowing down the amino acid mutational hotspot within these two regions. Table 1 shows the major mutations in these domains, the amino acid sequence undergoing mutation specifying the specific amino acid undergoing replacement, with respect to the original structure, giving rise to different variants. We have considered different variants with respect to S-protein and orf 1a/b as suggested in various literature and summarized in table 1.

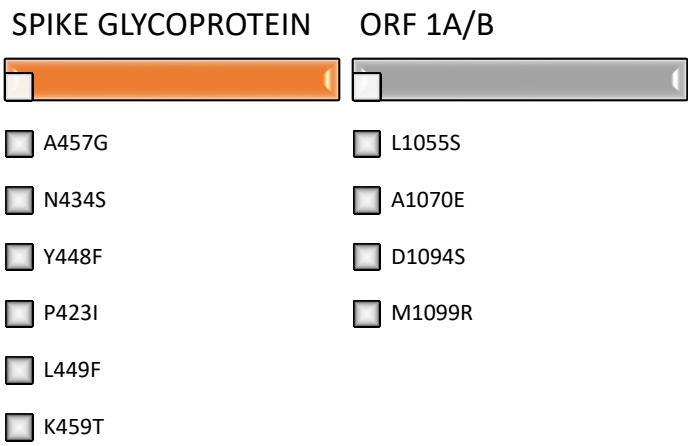


TABLE 1: AMINO ACID HOTSPOT MUTATION

Dipeptidyl-peptidase 4 (DPP4) acts as a receptor protein for the MERS-CoV ligand protein (Figure 1). Table 2 deciphers the protein-protein docking result, which was done using the HEX8.0 docking tool to find out the E-total and the number of hydrogen bonds involved in docking. Table 2 (a) depicts that the S-protein of the novel MERS-CoV virus involves more hydrogen bonds in binding with its receptor as compared to the variants. On the other hand, table 2 (b) shows that the orf 1 a/b mainly differs in its E-total value from the variants.

ORIGINAL S-PROTEIN	A457G	N434S	Y448F	P423I	L449F	K459T
H-BONDS: 103	H-BONDS: 69	H-BONDS: -1	H-BONDS: 69	H-BONDS: 69	H-BONDS: 69	H-BONDS: 69
E-TOTAL: 0.52	E-TOTAL: -320.53	E-TOTAL: 0.52	E-TOTAL: -320.44	E-TOTAL: -320.31	E-TOTAL: -320.42	E-TOTAL: -326.71

TABLE 2(a): HEX 8.0 Protein-protein docking result with respect to S-Protein

ORIGINAL ORF 1a/b	L1055S	A1070E	D1094S	M1099R
H-BONDS: 126	H-BONDS: 126	H-BONDS: 126	H-BONDS: 126	H-BONDS: 126
E-TOTAL: -122.91	E-TOTAL: -122.72	E-TOTAL: -122.92	E-TOTAL: -121.69	E-TOTAL: -122.98

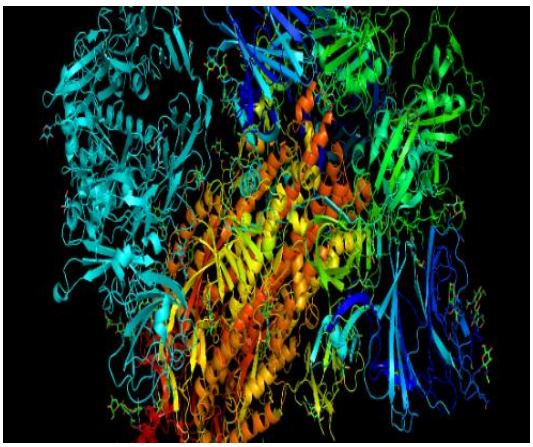
TABLE 2(b): HEX 8.0 Protein-protein docking result with respect to Orf 1a/b

The PDB form of DPP4 as a receptor and S-protein along with Orf 1a/b as a ligand was studied through the HDOCK tool to find out the value and position of the amino acid binding, along with its RMSD (root mean square division) value. The model with the minimum RMSD value was considered for further analysis. Table 3 summarises the docking results, showing the binding energy (BE) of different variants with the DPP4 receptor. The final result indicates that the L449F variant of S-Protein and the original Orf 1a/b region had the highest DPP4 receptor binding, indicating higher pathogenicity.

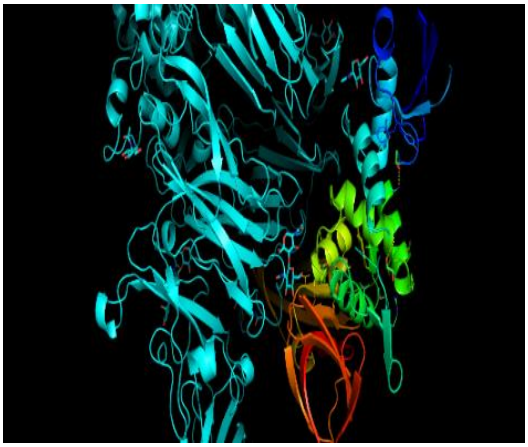
LIGANDS	AMINO ACID POSITION		AMINO ACID RESIDUE		CHAIN		BINDING ENERGY	DOCKING SCORE	RMSD VALUE
	R	L	R	L	R	L			
S-Protein	932	173	GLN	TYR	C	A	4.973	-374.06	373.34
A457G	481	246	LYS	LEU	B	A	4.973	-362.08	320.18
N434S	20	133	NAG	ASP	B	A	4.946	-360.32	320.27
Y448F	922	190	ASN	LYS	C	A	4.946	-374.06	373.34
P423I	25	169	NAG	ASN	B	A	4.919	-374.06	373.34
L449F	441	166	LYS	TYR	B	A	4.987	-360.32	320.27
K459T	937	181	SER	PRO	C	A	4.967	-374.06	373.34
Orf1a/b	1706	1	VAL	NAG	A	E	4.997	-357.30	80.15
L1055S	1083	487	GLY	ASN	C	B	4.949	-410.44	319.64
A1070E	259	529	CYS	ILE	C	B	4.992	-422.28	316.60
D1094S	47	549	TYR	GLU	C	B	4.972	-422.28	316.60
M1099R	259	529	CYS	ILE	C	B	4.993	-422.28	316.60

TABLE 3: HDOCK result

The PDB format extracted from the HDOCK tool was analysed via the PyMOL tool, which shows amino acid binding in different variants of MERS CoV.



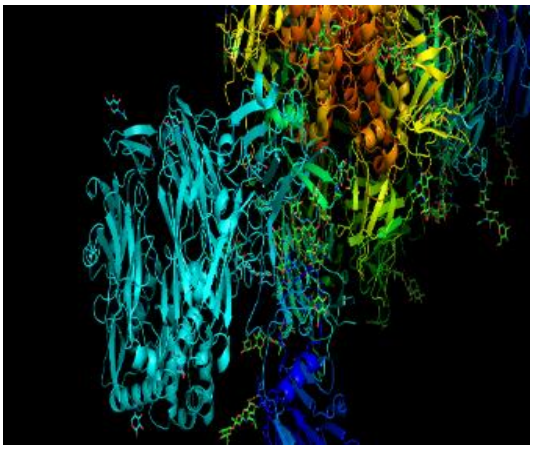
1(a)



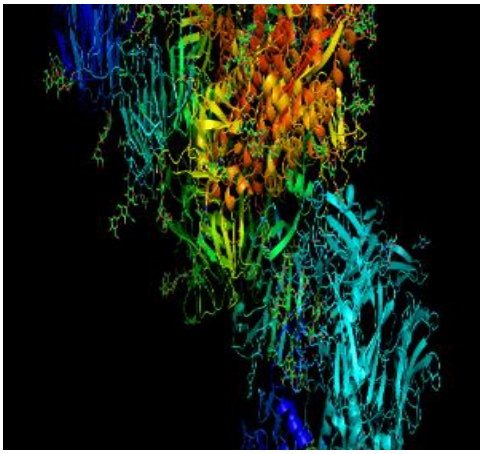
1(b)

Figure 1: **Original binding of MERS CoV with the DPP4 receptor** Fig. 1 a) depicts binding of S-protein with the DPP4 receptor whereby a sky blue ribbon designates the S-protein ligand and a yellow ribbon is the A chain of the receptor. Fig 1 b) shows the binding of Orf 1a/b with the DPP4 receptor, where the sky blue ribbon is Orf 1a/b while the orange ribbon is the receptor.

Figure 2 and Figure 3 respectively depict MERS CoV Orf 1a/b variant attaching to the DPP4 receptor and MERS CoV S-protein variants to the DPP4 receptor, respectively.



2(a)



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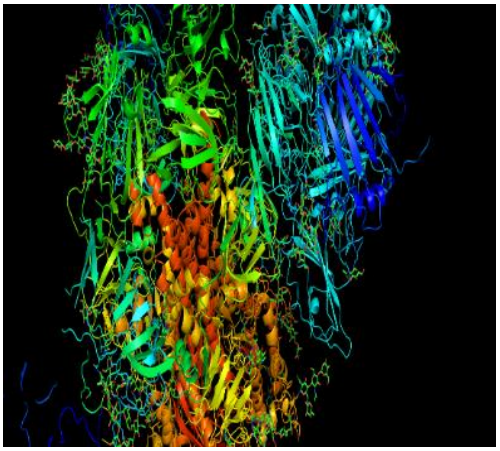
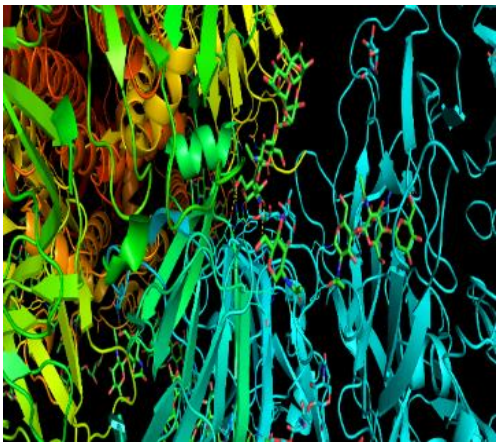
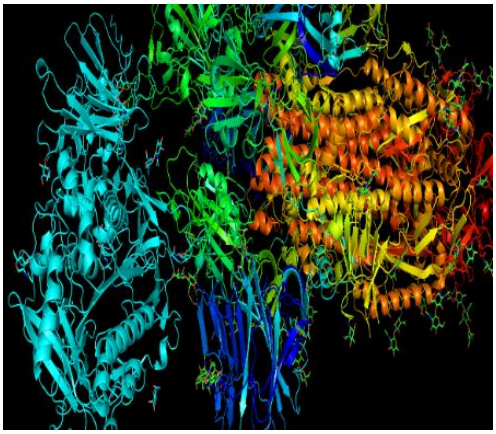


2(c)

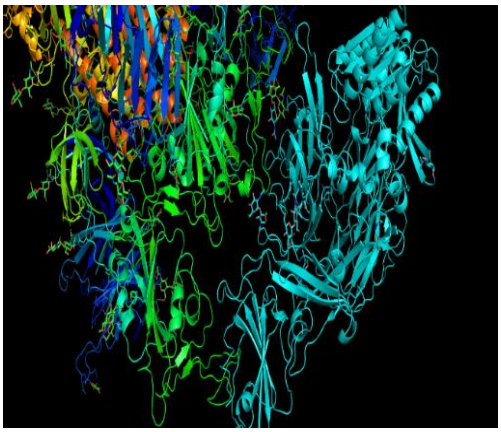


2(d)

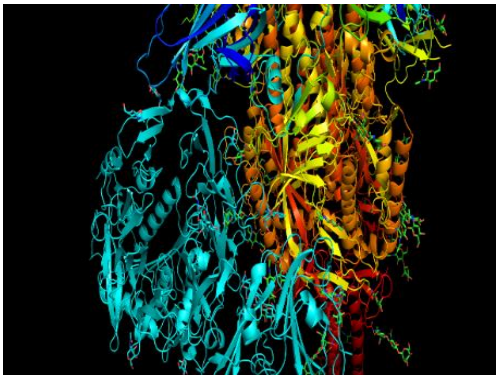
Figure 2: **MERS CoV Orf 1a/b variant binding to DPP4 receptor** Fig. 2 a) shows binding of the L1055S variant of Orf 1a/b, where the blue ribbon represents the ligand Orf 1a/b variant and the green ribbon represents the receptor DPP4. Fig. 2 b) represents the binding of the A1070E variant of Orf 1a/b where the sky blue ribbon is the ligand of the Orf 1a/b variant and the green ribbon depicts the receptor DPP4. Fig 2 c) represents the binding of D1094S variant of Orf 1a/b where sky blue ribbon is of ligand Orf 1a/b variant and yellow ribbon depicts receptor DPP4. Fig 2 d) shows binding of M1099R variant of Orf 1a/b where sky blue ribbon is of ligand Orf 1a/b variant while green ribbon depicts receptor DPP4.



3(a)



3(b)



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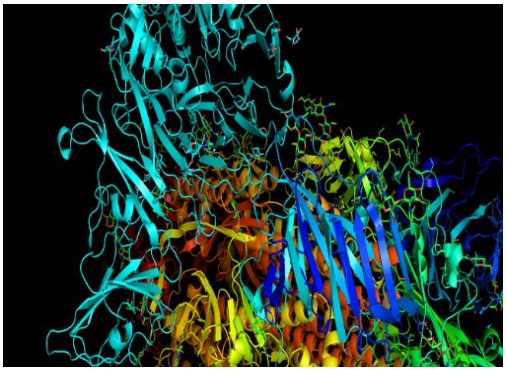
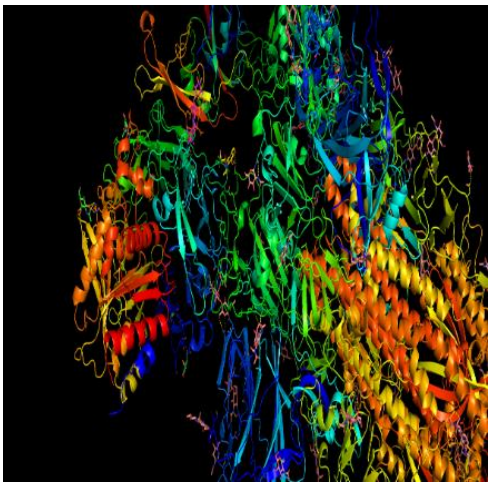
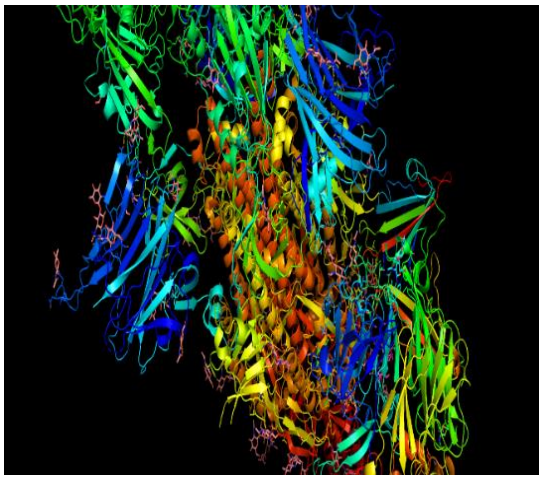


3(d)

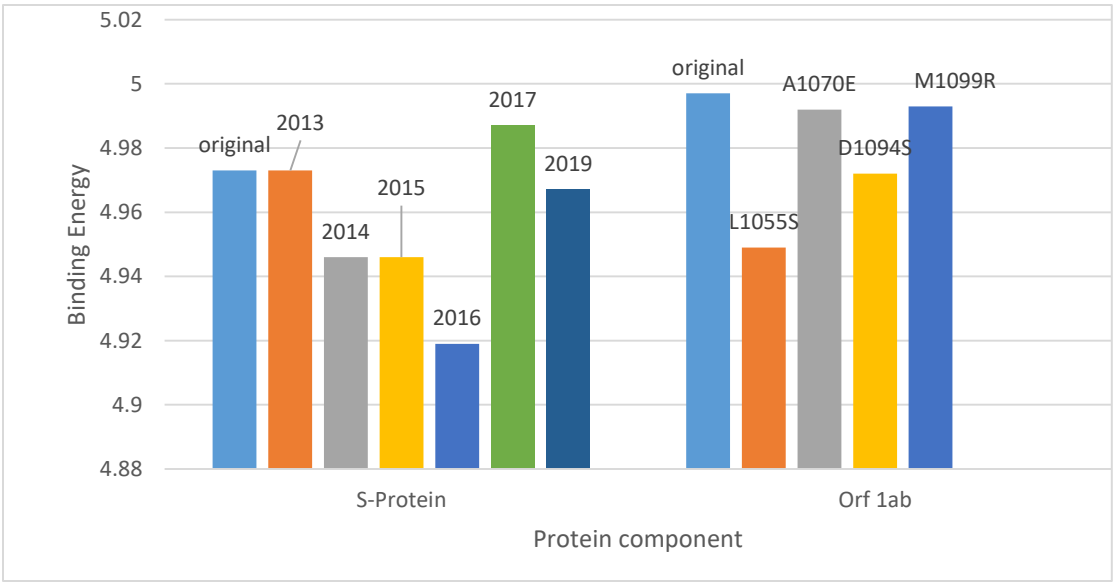


3(e)



3(f)

Figure 3: **Binding of MERS CoV S-protein variants to the DPP4 receptor** Fig. 3 a) shows binding of mutated S-protein in 2013 in sky blue to the DPP4 receptor in green. Fig. 3 b) depicts the binding of mutated S-protein with the DPP4 receptor in the year 2014. Fig. 3 c) depicts the binding of mutated S-protein in the year 2015 with the DPP4 receptor in orange. Fig. 3 d) depicts the binding of mutated S-protein in the year 2016 with the DPP4 receptor in a dark blue color. Fig. 3 e) depicts the binding of a mutated S-protein in the year 2017 in sky blue with the DPP4 receptor in orange. Fig. 3 f) depicts the binding of mutated S-protein in the year 2019 with the DPP4 receptor in green.



Graph 1: The graph outlines that the original Orf 1a/b (BE = 4.997) shows strong binding with the DPP4 receptor, followed by the 2017 variant of S-Protein (BE = 4.987). The other variants of Orf 1a/b also showed promising binding with the receptor, indicating its high infectivity rate.

Our studies showed that the amino acid binding remains in the variants of MERS CoV differed significantly in different variants in comparison to its wild-type structure. Major mutations in S-proteins were found as A457G, N434S, Y448F, P423I, L449F, and K459T. The major mutations found in MERS CoV Orf 1a/b are L1055S, A1070E, D1094S, and M1099R. The amino acid sequence majorly involved in mutation within the S-protein ranged from 420 to 460, while in the case of orf 1a/b, it was from amino acid 1050 to 2000. Figure 4 gives a comparative account of the different variants of S protein and Orf 1a/b and their binding efficiency with the DPP4 receptor, which is an indication of the infectivity potential of these variants and hence necessitates a more crucial therapeutic intervention.

CHAPTER 5- CONCLUSION

The vaccine development for the virus mainly targets the antigenic regions in the S protein and Orf 1a/b regions, which are often mutated in successive rounds of epidemics, rendering the pre-existing vaccines ineffective. Our study has revealed stretches of regions prone to mutations and also conserved sequences within these crucial domains of the virus essential for its infectivity, which do not mutate significantly. By studying the hotspot mutational regions in the surface-expressed viral gene products, better vaccines, and more efficient therapeutics can be developed to prevent future pandemics. The E-total and binding energy show that the original Orf 1a/b and 2017 variants of S-protein are more severe variants that lead to structural changes while replicating inside the host and could also lead to a further pandemic if the mutation rate doesn't subside. Our research also indicates that devising vaccines that are effective for severe variants may in fact cause a propensity for selective propagation of mutant strains that are less severe and hence may reduce mortality rates in infected individuals.

CHAPTER 6- REFERENCES

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