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Original Research Article

# Unveiling the secrets of fowl adenovirus: A bioinformatics odyssey of phylogenetic analysis, structure prediction, ADMET analysis, molecular docking, and molecular dynamic simulation

**Muhammad Danish Mehmood[1]*, Huma Anwar Ul-Haq[1], Faiza Khan[2], Abdul Rasheed Shaukat[3], Yasir Amin[4], Rabia Habib[1], Nusrat Tariq[5]**

[1]Dept. of Microbiology, Ottoman Pharma Immuno Division, Lahore, Pakistan
[2]The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[3]Poultry Production Department, Sabir's Poultry Pvt. Ltd, Sabroso, Pakistan
[4]Veterinary Research and Disease Investigation Center, Abbottabad, Pakistan
[5]M. Islam Medical & Dental College, Gujranwala, Pakistan

## Abstract

**Background:** The project abstract outlines the significant genetic diversity and structural variability exhibited by Fowl Adenovirus (FAdV) isolates. 100 liver samples from suspected Adenovirus-infected bird samples underwent virus isolation through homogenization and filtration.
**Materials and Methods:** Polymerase Chain Reaction (PCR) was utilized to amplify a specific segment of the viral DNA, which was then sequenced and analyzed for phylogenetic relationships. The physicochemical properties of the proteins from FAdV serotypes 4, 8, and 11 were analyzed, along with structural modelling.
**Results:** DHEA was identified as a suitable ligand for molecular docking, and docking studies were performed to explore interactions between the ligand and the viral proteins. ADMET analysis was conducted to assess potential drug-like properties and toxicity of the compounds. Ultimately, effective inhibitors were identified based on docking scores and molecular properties, highlighting potential avenues for therapeutic development against fowl adenoviruses.
**Conclusion:** This diversity is crucial in the development of vaccines and the design of antiviral drugs. Identifying potential drug targets and variations in ligand binding affinities underscores the importance of considering genetic diversity when devising immunophylactic and therapeutic strategies. The abstract emphasizes the need for future research to leverage these findings to deepen our comprehension of FAdV genetic diversity and enhance disease control measures by developing potent and effective homologous vaccines.

**Keywords:** Fowl adeno virus, Hexon Protein Structure, Molecular Docking, Phylogenetic analysis.

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## 1. Introduction

The Aviadenovirus, commonly found in chickens worldwide, is a significant member of the Adenoviridae family.[1] The classification of Fowl Adenovirus (FAdV) is primarily determined by analyzing the highly variable region of the L1 loop in the hexon gene. Additionally, the fiber gene of the virus also plays a role, albeit to a lesser extent, in the classification process. It is categorized into five species groups (FAdV-A to FAdV-E) and 12 serotypes (FAdV-1 to 8a and 8b to 11) based on their restriction enzyme and cross-neutralization test, determined through the hexon gene sequence analysis.[2] The non-enveloped, double-stranded DNA genome of FAdV is approximately 43-45 kb. These infections were initially identified in the latter part of the 20th century.[3]

The DNA of FAdV has a non-enveloped structure and consists of three main proteins: hexon, penton, and fiber. The FAdV genome encodes ten major structural proteins in the virion and 11 nonstructural proteins, which include E1A, E1B, DBP [E2A], ADP [E3], E4, 52/55 K, pIVaII, pol, EP,

*Corresponding author: Muhammad Danish Mehmood
Email: drdanishmehmood@gmail.com

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33 K, and 100. This detailed structure provides a comprehensive understanding of the virus.[4] Recently, investigation of the protein genes encoded for FAdV virulence determinant and infectivity was of significant interest to many researchers worldwide toward developing a safe and effective vaccine against the disease.[5,6] It was suggested that fiber and hexon genes play a significant role as a virulence determinant of FAdV infection.[7,8] Fowl adenovirus (FAdV) species mainly focused on the antigenicity of the virus, but the role of Structural proteins on the infectivity in host cells remains scanty.[9]

Exploring protein genes encoding FAdV virulence determinants and infectivity has captured researchers' interest in creating a safe and potent vaccine against the disease. There is a suggestion that the fiber and hexon genes play a pivotal role as virulence determinants of FAdV infection.[10] While previous studies have predominantly focused on the virus antigenicity, there is a notable lack of understanding regarding the impact of structural proteins on host cell infectivity.

The hexon protein is a significant component of the adenovirus capsid. It is composed of 240 non-vertex capsomeres, which form homotrimer structures. This arrangement gives the capsid a pseudo hexagonal shape with a triangular top superimposed on the base. It is important to note that the size of hexon molecules vary between different species and serotypes of Fowl Adenoviruses (FAdVs).[11]

The hexon protein's structure comprises variable loops and conserved pedestal sections. The variable loops, including L1, L2, and L4, are exposed on the outside surfaces and contain type-specific epitopes. The conserved pedestal sections, P1 and P2 are inside the varion. The highly conserved L3 loop, hidden internally, stabilizes the contact between the P1 and P2 conserved areas.[12]

The amino acid variations, including mutations, deletions, and additions, are frequently observed in the three intertwined loops due to high immunological selective pressures for neutralization with antibodies.[13,14] An in-depth analysis of the hypervariable regions (HVRs) in the hexon gene reveals that the L1 loop comprises HVRs 1–4, each with varying lengths. Specifically, HVR1 is 191 base pairs long, HVR2 is 50 base pairs long, HVR3 is 90 base pairs long, and HVR4 is 18 base pairs long. Significant variations in the sequence of HVRs are observed between FAdVs types, although they remain constant for every species. Notably, the L1 loop regions exhibit the highest sequence variability and are the most extended loop in the protein. This intricate folding structure serves as the location of specific receptors.[15]

Current FAdV antigenicity and pathogenicity research focuses on molecular studies, particularly hexon and fiber proteins.[9] Fowl adenovirus (FAdV) species have been the subject of extensive research, particularly about the antigenicity of the virus. However, there remains a lack of comprehensive understanding regarding the impact of structural proteins on the virus ability to infect host cells.[10] This highlights the need for further investigation into the specific mechanisms by which these structural proteins may influence the infectivity of FAdV in host cells. Fowl adenovirus infections significantly threaten the poultry industry, leading to various clinical diseases in chickens. The impact of these infections is substantial, resulting in high mortality rates and reduced productivity, leading to significant economic losses.[16]

FAdV is responsible for causing various diseases, including the hepatitis-hydropericardium syndrome (HHS) epidemic, inclusion body hepatitis (IBH), respiratory tract infections, and gizzard erosions, all of which affect the liver.[17,18]

Epidemiological studies have established that Inclusion body hepatitis (IBH) is commonly attributed to FAdV serotypes 2 or 8a, 8b, and 11.[19] IBH is characterized by a sudden onset of high mortality, peaking 3–4 days post-infection (pi) and returning to normal on day 5, although occasionally persisting for 2–3 weeks.[20] While mortality rates are relatively low, affected chickens display clinical signs of depression, ruffled feathers, and reduced feed consumption prior to succumbing to the disease.[21] Gross lesions of IBH are typified by hepatic necrosis and inflammation, featuring friable, swollen, pale, and petechial hemorrhages in the liver of afflicted chickens.[22] Hemorrhagic Hepatovirus Syndrome (HHS), caused by FAdV serotype 4, is characterized by sudden death in broiler chickens, with mortality rates ranging from 20% to 80%.[23,24] This condition predominantly affects commercial broiler flocks aged 3–5 weeks, characterized by lesions of a swollen pericardial sac filled with straw-colored fluid and swollen, friable liver.[25,26] Gizzard erosion, attributed to FAdV serotypes 1 and 8b, has been consistently reported in broiler and layer chickens in various countries. The disease adversely impacts the performance of broiler flocks, resulting in reduced body weight gain, high mortality rates of up to 80%, and an increased condemnation rate at the slaughterhouse.[27] Furthermore, a decrease in egg production, coupled with egg malformation, has been observed in layer chickens infected with the virus.[28]

In a recent computer-based study, researchers used bioinformatics methods, molecular simulation, and target identification to investigate potential new options for developing an influential vaccine or drug to combat Fowl Adenoviruses (FAdV) shortly. This promising research could lead to significant advancements in the field.

## 2. Materials and Methods

### *2.1. Sample collection*

Using the statistical formula (n = Z2 P (1-P) / d2), 30 g of liver was collected in a sterile polythene-labelled bag from each of 100 suspected Adenovirus-infected broilers/breeder

flocks reported from all across Pakistan and transported to Ottoman Pharma (Immuno Division), where it was stored at -20°C until further use.

*2.2. Virus isolation*

10 % liver homogenate was prepared in 0.9 % Normal saline (Zeesol Pharma-Pakistan) solution in an electric homogenizer (B. Braun-Germany). The suspension was centrifuged at 2°C and 3000 rpm for 2 minutes, and the supernatant was collected for filtration. The clear supernatant was then passed through a 0.2 µm size filter (Sartorius-Germany) and admixed with 1 % of pen strep (Gibco-USA) in the final concentration.[29]

*2.3. Polymerase chain reaction (PCR)*

Favorgen (Taiwan) extraction kit was used to extract viral DNA from the liver homogenate of FAdV-positive samples. For Polymerase chain reaction (PCR), 899bp- sized partial hexon gene of FAdV primer Forward: 5' CAARTTCAGRCAGACGGT 3' and Reverse: 3' TAGTGATGMCGSGACATCAT 5' was used. The total volume was 25ul, in which 12ul master mix, 7ul nuclease-free water, 1 ul each forward and reverse primer, and 4ul DNA template were added. The DNA was amplified with an initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 45 sec, annealing at 59°C for 45 sec, and extension at 72°C for 45 sec followed by 30 cycles. The final extension was performed at 72°C for 10 minutes in a Veritii thermocycler (Applied Biosystems-Thermo Fisher-USA) followed by Gel Electrophoresis (Major Science-Taiwan) and band visualization via UV Transilluminator (Vilber Lourmat-France) as can be reviewed in.

*2.4. Phylogenetic analysis*

The PCR amplicon was sent to APICAL SCIENTIFIC SDN. BHD., Malaysia, for sequencing. Each sequence was subjected to a Basic Local Alignment Search Tool (BLAST) to compare it with similar sequences submitted to the National Center for Biotechnology Information (NCBI). The FAdV sequence isolates submitted to the NCBI gene bank are now available. Moreover, the phylogenetic comparison was conducted by constructing a maximum likelihood phylogenetic tree in Sequence Analysis MEGA 11 Software using the MUSCLE alignment algorithm.

*2.5. Protein selection*

The protein sequences of fowl adenovirus serotypes 4, 8, and 11 were extracted from the UniProt database in FASTA format. The Expasy ProtParam tool was used to analyze these serotypes' physicochemical properties and secondary structure. Initially, homology modelling was performed on Indigenous isolates to predict their three-dimensional structures. Following this, threading and ab initio modelling techniques were utilized to compare the structural characteristics of FAdV-4, FAdV-8, and FAdV-11.

*2.6. Selection and retrieval of ligand*

A literature study conducted through Google Scholar and PubChem found that DHEA (Dehydroepiandrosterone) is a ligand compound suitable for molecular docking of fowl adenovirus 4, 8, and 11. Synthetic mixtures of this compound are available from PubChem. The 2D compound structure was retrieved and converted into 3D, then visualized using PyMOL 2.5.7.

*2.7. Molecular docking*

The molecular docking results for fowl adenovirus serotypes 4, 8, and 11 were obtained using PyRx 0.8. Subsequently, PyMOL was employed to analyze the protein-ligand complex, aiming to understand the interaction between the receptor and inhibitor and the binding sites of the target protein.

*2.8. ADMET analysis*

After thoroughly analyzing the docking results of fowl adenovirus serotypes 4, 8, and 11, the study identified drug similarity and toxicity characteristics using SwissADME. These findings are valuable for calculating important drug-like properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET). Additionally, they can be used to predict lead similarity between mutagenicity and carcinogenicity.

*2.9. Lead identification*

The most effective inhibitors were identified based on docking score, ligand-protein interactions, and toxicity analysis studies, including Molecular Weight (MW), Hydrogen Bond Donor (HBD), Hydrogen Bond Acceptor (HBA), partition coefficient logP, polar surface area (PSA), rotatable bonds, rings, Blood-Brain Barrier, and Ames Toxicity. Compounds demonstrating the most favorable affinity, high structural similarities, and best interaction were selected as potential inhibitors of Scm.

## 3. Results

The reference isolates for the indigenous isolates FAdV-4, FAdV-8, and FAdV-11 were obtained from the NCBI database. At the same time, the accession IDs for the indigenous isolates are as follows: FAdV-4 (PP341297, OR631197, OR166260, OR239206, OR631195), FAdV-8 (PP236758, PP857842, UFMG2014, PP354074, PP993463), and FAdV-11 (PP695181, PP982455, PP982457, PP993466, OR631196).

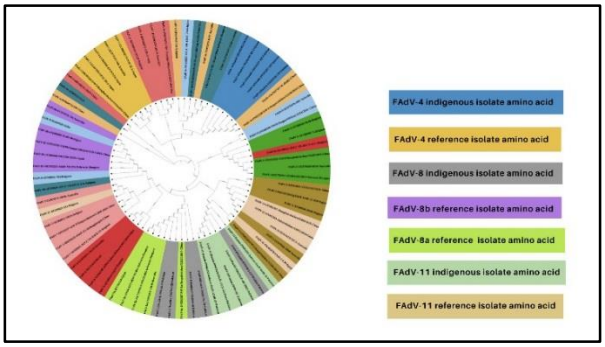


**Figure 1:** Phylogenetic analysis of Indigenous isolate of fowl adenovirus of serotype 4, 8, and 11 based on amino acid sequence. Sixty references representing isolates of FAdVs serotype (1 to 8a and 8b to 11) from different countries were analyzed. The phylogenetic tree was generated by MEGA 11. The tree was determined by bootstrapping of multiple sequence alignment.

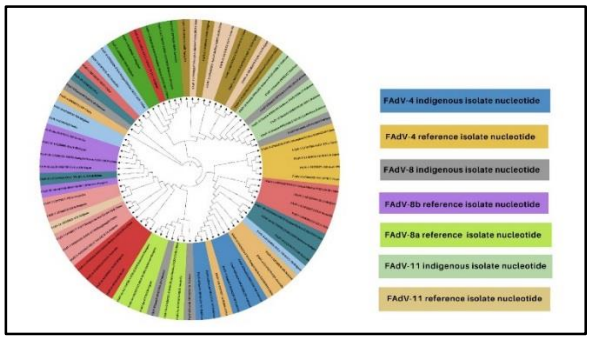


**Figure 2:** Phylogenetic analysis of Indigenous isolate of fowl adenovirus of serotype 4, 8, and 11 based on nucleotide sequence. Sixty references representing isolates of FAdVs serotype (1 to 8a and 8b to 11) from different countries were analyzed. The phylogenetic tree was generated by MEGA 11. The tree was determined by bootstrapping of multiple sequence alignment.

In the phylogenetic analysis, a tree was constructed based on nucleotide and amino acid sequences to determine the positioning of indigenous isolates of serotypes 4, 8, and 11 within the broader context of reference isolates from FAdV-1 to FAdV-8a and FAdV-8b to FAdV-11. The amino acid sequence of serotype 4 indigenous isolates closely resembles serotypes 4 and 10 of the reference isolate. Four amino sequences of serotype four share a clade value of 0.00000333, similar to serotypes 4 and 11, indicating genetic similarity. However, one sequence (Accession ID: OR631195) has a much larger clade value of 0.01079503 and is closely related to serotype 4, suggesting significant genetic divergence from the others. Similarly, serotype 8 indigenous isolates are related to the serotype 8a region of the reference isolates. One amino acid sequence of serotype 8 is associated with the indigenous isolate of serotype 11, all of which have a clade value of 0.0000033. Serotype 11 resembles the serotype 2 region of the reference isolate and the serotype 8 region of the indigenous isolate, with the same clade value of 0.00000333.

For nucleotide sequences, serotype 4 indigenous isolates is related to the serotype 4 region of the reference isolates. The sequence with Accession ID: OR631195 has a clade value of 0.00491101, more significant than that of Accession ID: OR239206 (0.00000238). The other three isolates' clade values are similar (0.00000119).

In serotype 8, two sequences resemble the serotype 8a region, while two others are related to serotype 11 of indigenous isolates. One sequence related to serotypes 10 and 4 of the reference isolate. The clade value for two isolates is 0.00000119, while the others vary: Accession ID: PP354074 has a clade value of 0.81108509, Accession ID: PP236758 has a clade value of 0.00939727 and Accession ID: PP857842 has a clade value of 0.0000015. These differences in clade values reflect varying degrees of genetic distance.

Serotype 11 resembles the serotype 8 region of the indigenous isolate and the serotype 2 region of the reference isolate. While four isolates share the same clade value of 0.00000119, one isolate (Accession ID: PP695181) has a more considerable clade value of 0.00233975.

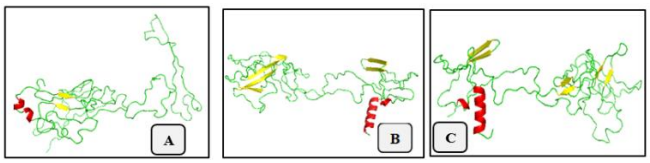


**Figure 3:** 3D Hexon protein structure of fowl adenovirus serotype 4 Accession # PP341297 through **A:** Homology modelling, **B:** Ab initio (Rosetta) and **C:** Threading (I-taser)

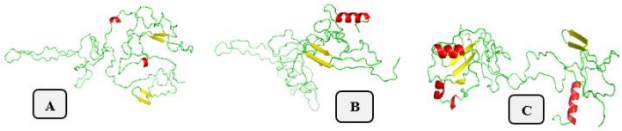


**Figure 4:** 3D Hexon protein structure of fowl adenovirus serotype 8 Accession # PP236758 through; **A:** Homology modelling; **B:** Ab initio (Rosetta) and **C:** Threading (I-taser)

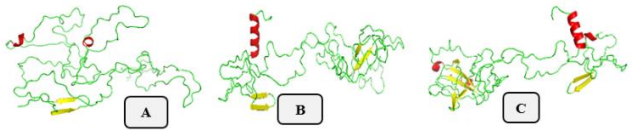


**Figure 5:** 3D Hexon protein structure of fowl adenovirus serotype 11 Accession # PP695181 through; **A:** Homology modelling; **B:** Ab initio (Rosetta) and **C:** Threading (I-taser)

The protein structures of fowl Adenovirus serotypes 4, 8 and 11 were predicted using three different methods: homology modelling, ab initio, and threading. Each method resulted in distinct structures. For fowl adenovirus serotype 4 accession # PP341297, the number of amino acids is 276 and the molecular weight is 30525.80. The homology model consisted of two alpha-helices and two beta-sheets. In

comparison, the ab initio model (Rosetta) and the threading model (i-Tasser) predicted structures with six beta-sheets and two alpha-helices, as shown in **Figure 3.** Similarly, fowl adenovirus serotype 8, accession # PP236758, has 288 amino acids and a molecular weight 31561.17. The homology model included four beta-sheets and two alpha-helices, the ab initio model featured five beta-sheets and four alpha-helices, and the threading model showed two beta-sheets and one alpha-helix, as depicted in **Figure 4**. Furthermore, fowl adenovirus serotype 11 accession # PP695181 has 283 amino acids and a molecular weight is 31030.68. The homology model consisted of two beta-sheets and two alpha-helices, the ab initio model had seven beta-sheets and four alpha-helices, and the threading model displayed four beta-sheets and one alpha-helix, as illustrated in **Figure 5**.

ExPASy ProtParam software was used to analyze the physicochemical properties of the three predicted structures for serotypes 4, 8, and 11. The analysis showed consistent properties across different prediction methods for each serotype. The extinction coefficient, which measures how strongly a protein absorbs or reflects light at a specific wavelength, ranged from 4.0 to 24.0. The table shows that FAdV-4 has an extinction coefficient of 1.570, FAdV-8 has 1.246, and FAdV-11 has 1.396. The half-life, indicating how long a protein can survive in different environments, was found to be 1.3 hours in mammalian reticulocytes (in vivo), 3 minutes in yeast (in vivo), and 3 minutes in Escherichia coli (in vivo) for FAdV-4 and FAdV-11. In comparison, it was 100 hours in mammalian reticulocytes (in vivo), 20 hours in yeast (in vivo), and 10 hours in Escherichia coli (in vivo) for FAdV-8. According to the half-life results, serotype 8 showed better stability. An instability index of less than 40 is considered ideal, indicating protein stability. FAdV-4 had an instability index of 26.37 (Stable), FAdV-8 had 26.11 (Stable), and FAdV-11 had 17.70 (Stable). The aliphatic index, which indicates the positive factor for thermo stability, ranged from 61.09 to 83.59. FAdV-4 had an aliphatic index of 65.69, FAdV-8 had 68.72, and FAdV-11 had 70.32. The GRAVY (Grand Average of Hydropathicity) score, which assesses protein hydrophobicity and hydrophobicity, indicated that a negative value suggests the protein is hydrophilic. FAdV-4 had a GRAVY score of -0.536 (Hydrophilic), FAdV-8 had -0.406 (Hydrophilic), and FAdV-11 had -0.371 (Hydrophilic). Based on these findings, we selected serotype 8 for further analysis due to its superior physiochemical properties compared to serotype 4 and serotype 11 as explained in **Table 1**.

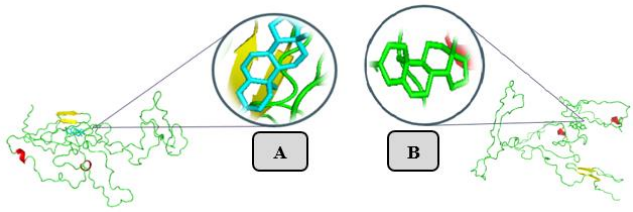


**Figure 6:** Hexon Protein and Ligand Dehydroepiandrosterone (DHEA) ID (CID-5881) molecule complex **A:** serotype 4 and **B:** serotype 8 &11

The DHEA (Dehydroepiandrosterone) ligand was chosen based on data from PubChem and relevant literature. Molecular docking was carried out using the hexon protein, which is the sole protein considered. The docking results revealed that serotype 4 produced ten molecules with ligand ID (hexon_5881_uff_E=874.50) exhibiting varying binding affinities, ranging from -5.6 to -6.9. Serotypes 8 and 11 yielded similar results, with nineteen molecules and two different Ligand IDs. Serotype 8 had ten molecules with the Ligand ID (hexon_5881_uff_E=874.50) and nine with the Ligand ID (hexon_5881_uff_E=595.59), displaying binding affinities ranging from -6.5 to -7.8 and -7 to -8.7, respectively. Serotype 11 had ten molecules with the Ligand ID (hexon_5881_uff_E=595.59) and nine with the Ligand ID (hexon_5881_uff_E=874.50), showing binding affinities with similar ranges. The Ligand ID (hexon_5881_uff_E=874.50) with the highest binding affinity of -7.8 was selected due to its presence in each serotype.

**Table 1:** Physiochemical properties of fowl adenovirus Indigenous isolate

| Properties | FAdV-4 | FAdV-8 | FAdV-11 |
|---|---|---|---|
| **Extinction Co-efficient Wavelength=280nm** | TRP residue found<br>Abs 0.1% 1.570<br>Ex. Coefficient = 47790 | TRP residue found<br>Abs 0.1% 1.246<br>Ex. Coefficient = 39310 | TRP residue found<br>Abs 0.1% 1.396<br>Ex. Coefficient = 43320 |
| **Half-Life** | 1.3 hrs. (Mammalian reticulocytes, in vivo)<br>3 min (Yeast, in vivo)<br>3 min (*E. coli*, in vivo) | 100 hrs. (mammalian reticulocytes, in vivo)<br>20 hrs. (Yeast, in vivo)<br>Ten hrs. (*E. coli*, in vivo) | 1.3 hrs. (mammalian reticulocytes, in vivo)<br>3 min (Yeast, in vivo)<br>3 min (*E. coli*, in vivo) |
| **Instability >40** | 26.37 (Stable) | 26.11 (Stable) | 17.70 (Stable) |
| **Aliphatic Index Range=61.09 to 83.59** | 65.69 | 68.72 | 70.32 |
| **Gravity** | -0.536 (Hydrophilic) | -0.406 (Hydrophilic) | -0.371(Hydrophilic) |

The identical ligand IDs and corresponding similar canonical SMILES led to the same ADMET analysis results, as illustrated in **Figure 3.** The compound's ADMET properties were evaluated using SwissADME. The compound's structure, represented by the canonical SMILES notation (CC12CCC3C(C1CCC2=O)CC=C4C3(CCC(C4)O)C), was obtained from PubChem for this analysis.

The VaxiJen tool forecasts the antigenicity of a specific viral protein sequence, with a threshold value set at 0.4. AllerTOP determines the most likely route of exposure for tested proteins identified as allergens.

*3.1. Molecular dynamic simulation*

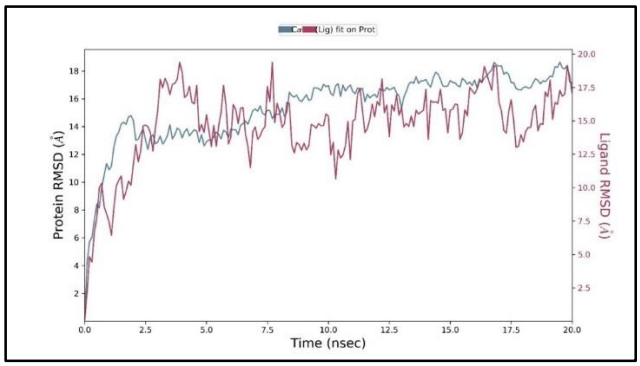


**Figure 7:** Root mean square deviation (RMSD) of the backbone atoms of hexon protein and the ligand with time. The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

The graph showed that the simulation spans 20 nanoseconds, which may be too short to accurately assess the complex's stability. The peak lines on the graph indicate the complex's stability, demonstrating minimal fluctuations and significant overlap, suggesting stability throughout the simulation, as shown in.

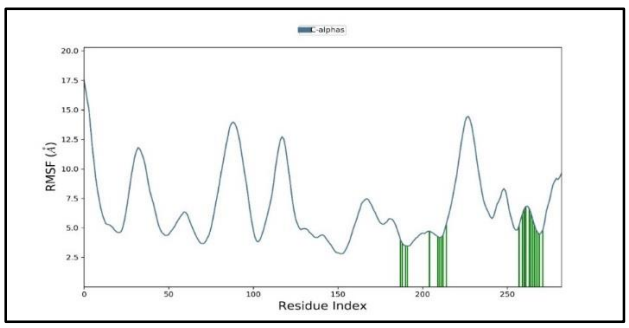


**Figure 8:** Residue-wise Root Mean Square Fluctuation (RMSF) of hexon protein.

This graph shows residue residue-wise RMSF value of protein bound to ligand; protein has 270 to 280 long residues, indicating fluctuation and interaction; the green shaded part shows residues' interaction with ligands as indicated in **Figure 8**.

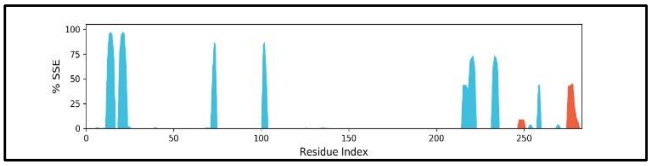


**Figure 9:** Protein Secondary Structure element distribution by residue index throughout the hexon protein structure. Red columns indicate alpha helices and blue columns indicate beta-strands

The graph showed the distribution of secondary structures in a hexon protein with blue columns indicating beta-strands and red columns indicating alpha-helices. The x-axis represents the residue positions, and the y-axis shows the percentage of secondary structure. Regions with tall blue or red columns are structured, forming either beta-sheets or alpha-helices, while gaps suggest flexible or unstructured areas of the protein.

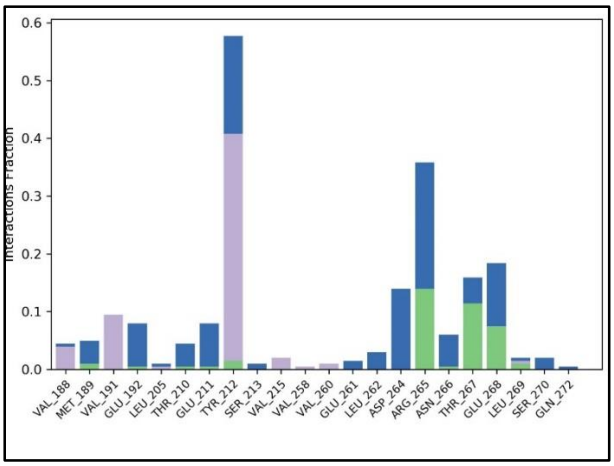


**Figure 10:** Hexon Protein and Dehydroepiandrosterone ligand molecule contact histogram

The graph shows the interaction fractions of various amino acid residues in a protein, indicating how consistently each residue is involved in interactions. High bars (e.g., TYR-212, ASN-266) suggest key interaction sites or hotspots. The bar's colors represent various interactions, such as hydrogen bonding or hydrophobic contact. This data helps identify crucial residues for the protein's function, essential for drug design or understanding molecular mechanisms.

## 4. Discussion

In this research study, we conducting a comprehensive analysis of the genetic and structural traits of indigenous fowl adenovirus (FAdV) isolates, explicitly focusing on serotypes 4, 8, and 11. Our primary objective was to gain a deeper understanding of the genetic diversity and distinctive structural features of FAdVs. By doing so, we aim to pave the way for developing more effective and targeted treatments for poultry diseases. This in-depth analysis will provide valuable insights into the molecular characteristics of these FAdV serotypes, which will be crucial for advancing poultry

disease management and control strategies. Mutations within the hypervariable regions (HVRs) have conferred the adenovirus with the ability to evade the host's immune response, as these regions play a critical role in antibody binding.[30] Additionally, the sequences encoded within the HVRs exhibit marked differentiation between species or types and among strains that infect distinct hosts. Analysis of the HVR1 region has elucidated that the amino acid sequence is host-specific to the respective adenovirus.[31]

### *4.1. Phylogenetic insights and genetic differences*

In the genetic analysis of the FAdV isolates, it was observed that most of them clustered within their respective serotypes, indicating genetic similarity. However, there were exceptions to this pattern. For instance, a specific FAdV-4 isolate (OR631195) demonstrated a significant genetic divergence compared to other FAdV-4 isolates. This suggests that this particular isolate may have evolved differently or undergone a recombination event (Niczyporuk and Czekaj, 2018). This finding aligns with previous studies that have also highlighted genetic variation within FAdV serotypes, emphasising the importance of ongoing surveillance to identify new strains that may exhibit varying levels of virulence or possess the ability to evade the host immune response.[32]

In our analysis of serotype 8, we observed that specific indigenous isolates share a closer genetic relationship with serotype 11 reference strains. This finding has significant implications for the efficacy of vaccines against these viruses. Additionally, the genetic similarity between serotype 11 isolates and regions of both serotype two and serotype 8 indicates the complex evolutionary relationships within FAdVs. This insight, as reported by Chavan, sheds light on the intricate dynamics of FAdV evolution and its potential impact on viral characteristics.[33]

### *4.2. Structural differences and their effects*

We have discovered substantial structural disparities using various computational methods to anticipate the 3D configurations of hexon proteins in the three serotypes.[34] These distinctions, particularly in the quantity of beta-sheets and alpha-helices, may impact the virus interaction with host cells and its ability to evade the immune system. The diversity in protein structure suggests that employing multiple computational models can offer deeper insights into the structure, which is valuable for developing effective vaccines or antiviral drugs.[35]

### *4.3. Ligand binding and molecular docking*

Our research findings from molecular docking indicate that the hexon protein of serotype 8 exhibits the strongest affinity for the DHEA ligand.[36] This suggests that it can potentially be a promising target for developing antiviral drugs.[37] The consistent binding affinities observed across different serotypes for similar ligands imply the presence of conserved binding sites. However, the variations in binding affinities between isolates suggest the need for personalized approaches to account for the genetic diversity of the virus.[38]

### *4.4. Molecular dynamics and protein-ligand interactions*

In our recent molecular dynamics simulations, we observed that the protein-ligand complexes exhibited remarkable stability throughout the simulation, as evidenced by consistent and low root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) values. This observation suggests that the protein structures bound to the ligands could serve as promising targets for drug development initiatives. However, it is essential to acknowledge that our simulations were relatively short, spanning only 20 nanoseconds. As a result, the dynamic behavior of these complexes over more extended time frames may not have been fully captured. Consequently, it is imperative to conduct future studies involving longer simulation times to validate and consolidate these preliminary findings.[39]

### *4.5. Study limitations and future directions*

Our research has gained valuable insights into the genetic and structural characteristics of Indigenous FAdV isolates. However, it is essential to acknowledge certain limitations that should be considered. Firstly, the short duration of our molecular dynamics simulations restricts our ability to fully comprehend the long-term stability of the protein-ligand complexes. Additionally, our analysis was based on a limited number of isolates, which may not provide a comprehensive representation of the genetic diversity of FAdVs across various geographical regions. Future research endeavours should encompass a broader spectrum of isolates and incorporate more extended simulation periods to address these limitations. This approach will enable us to acquire a more comprehensive understanding of the evolutionary dynamics and structural stability of FAdVs.

## 5. Conclusion

The study's findings underscore the significant genetic diversity and structural variability exhibited by Fowl Adenovirus (FAdV) isolates. Identifying potential drug targets and the variation in ligand binding affinities underscores the importance of considering genetic diversity when devising immunophylactic and therapeutic strategies. Moving forward, it is imperative for future research to leverage these findings to deepen our comprehension of FAdV genetic diversity. This will enhance disease control measures by developing potent and effective homologous vaccines.

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## 7. Conflict of Interest

None.

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