

### Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Comparative Studies of H5N1 Gene Segments with other Subtypes of Influenza- A Virus.

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#### ABSTRACT

Comparative genomics of H5N1 virus will help to analyze the evolution of subtypes of Influenza A viruses and to know the differences in their genes and surface proteins which could be useful in understanding their mode of action. In this study we have compared all gene segments and proteins of H5N1 with other types of influenza-A viruses such as H1N1, H2N2, H3N2 and H9N2. The comparative analysis revealed that H5N1 was phylogenetically closer to H9N2. Homology percentages of eight genes of H1N1, H2N2, H3N1, H5N1 and H9N2 were calculated. Average homology of genes of aligned segments in subtypes of H5N1, H9N1 was found to be 81.5%.



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#### INTRODUCTION

An influenza pandemic is a global outbreak of disease that occurs when a new influenza A virus appears or "emerges" in the human population, causes serious illness, and then spreads easily from person to person worldwide [1]. Past influenza pandemics have led to high levels of illness, death, social disruption, and economic loss [2]. Influenza viruses come under the class of orthomyxo-viridae family, having a single stranded negative sense, segmented RNA genome in an enveloped virian [3]. There are three types of Influenza viruses: A, B and C, differentiated by antigenic differences in their nucleoprotein and matrix protein. The genome of influenza consists 8 RNA segments and encodes 10 proteins including the internal structural proteins, nucleocapsid protein (NP), and the two matrix proteins (M1 & M2). The segmented nature of the genome also allows for the exchange of entire genes between different viral strains when they cohabitate the same cell [4].

The surface proteins hemagglutinin (HA) and neuraminidase (NA) extensively studied that are essential to the virus life cycle and the antigenic variations in the surface glycoproteins are used to subtype influenza A [5]. In addition, three of the influenza polypeptides are associated with RNA polymerase activity (PA, PB1, and PB2). The RNA binding non-structural protein (NS) contributes to viral pathogenicity and plays a central role in the prevention of interferon mediated antiviral response [6].

The H5N1 virus, an avian influenza virus (Bird flu) can cause illness in many species including humans by undergoing the process of Antigenic shift in some intermediate host, generally a pig or chicken[7]. H5N1 can be considered as a pandemic threat as it can move from areas where it is endemic to other parts of the world through migrating water fowl[8]. H5N1virus has mutated through antigenic drift into dozens of highly pathogenic varieties, but all currently belonging to genotype Z of avian influenza virus H5N1[9-10].

This infection of humans coincided with an epizootic (an epidemic in nonhumans) of H5N1 influenza in Hong Kong's poultry population and was capable of emerging in to a panzootic disease [11]. Populations tend to have more resistance to influenza B and C, because they only undergo antigenic drift and have more similarity with previous strains[12]. Sixteen H subtypes and nine N subtypes have been identified for influenza A virus. Only one H subtype and one N subtype have been identified for influenza B virus. The most common antigenic variants of influenza A virus are H1N1 and H3N2 [13].

This study consists of comparing all the gene segments and some important proteins of H5N1 virus with other types of Influenza A viruses like H1N1, H2N2, H3N2 and H9N2. Comparative genomics of H5N1 virus will help to analyze the evolution of these subtypes and to understand the differences in their genes and surface proteins which could be useful to comprehend their mode of action and genetic drifts among these viruses.



METHODS

#### **Data Mining**

All the gene sequences and amino acid sequences of all proteins of H5N1, H1N1, H2N2, H3N2 and H9N2 should be collected from the NCBI Public Database, which is maintaining a separate public genome and proteome database for all the Influenza viruses' i.e. A, B and C types of Influenza viruses. All the subtypes studied in our project work belong to Influenza A virus. All the genes should be sorted according to their corresponding virus subtypes[14].

#### Comparative study of genes and amino acid sequences

The analyzed genes and proteins should be compared to have a basic idea of their evolution and know the differences in their genes and proteins and how these differences are responsible for the change in their virulence or pathogenicity. By that we can know in which aspects H5N1 is different from other subtypes and which aspects it is similar. This comparative study was done using various tools available in JEMBOSS like PLOTCON, Pretty plot, Clustal W, BLAST P, BLAST N[15-17].

#### **Phylogenetic analysis:**

The phylogenetic analysis of the various subtypes of Influenza A virus will give the idea of ancestral relationships among the subtypes. The phylogenetic analysis of the 5 subtypes selected in our study sophisticated tool like NJplot (Neighbor Joining plot) used for a better understanding. Using this tool phylogenetic tree can be constructed for all the eight gene segments of the selected 5 subtypes.

#### **RESULTS AND DISCUSSION**

#### Multiple alignments of gene segments of Influenza-A subtypes

The eight genes of H1N1, H2N2, H3N1, H5N1 and H9N2 have been aligned by using the multiple alignment tool Clustal W and their percentage of homology is presented in Table (1). Based on homology percentages it was observed that H5N1 showed highest similarity with H9N2.The similarity regions of nucleotides in segment 2 of H1N1, H2N2, H3N2, H5N1 and H9N2 were analyzed by Pretty plot and was found that segment-2 Of H5N1 similar to H9N2 (figure 1).The aligned regions of these gene segments were merged using Plotcon for identification of conserved regions. Earlier it was reported as 87 conserved regions were identified in the influenza genome and in avian host strains, the segments NS, M show the most conservation (94.5%), followed by the PB1, NP, PA, and finally the PB2 segment. In the swine strains the M segment shows the most conservation followed by the segments NP, PB2, PA, and finally PB1[18].

#### ISSN: 0975-8585



Aligned Subtypes	Percentage homology of genes						Avg.(%)		
	PB2	PB1	ΡΑ	HA	NP	NA	М	NS	
H1N1 : H2N2	92 %	83 %	93 %	70 %	91 %	55 %	94 %	92 %	83.7
H1N1 : H3N2	89 %	82 %	89 %	58 %	87 %	60 %	92 %	89 %	80.7
H1N1 : H5N1	83 %	83 %	85 %	66 %	86 %	81 %	90 %	74 %	81.0
H1N1 : H9N2	83 %	83 %	85 %	63 %	84 %	57 %	88 %	89 %	79.0
H2N2 : H3N2	93 %	89 %	93 %	60 %	92 %	88 %	94 %	93 %	87.7
H2N2 : H5N1	83 %	90 %	84 %	71 %	84 %	56 %	89 %	72 %	78.6
H2N2 : H9N2	83 %	88 %	83 %	63 %	82 %	84 %	88 %	87 %	82.2
H3N2 : H5N1	83 %	86 %	83 %	58 %	83 %	56 %	87 %	75 %	76.4
H3N2 : H9N2	82 %	86 %	82 %	59 %	81 %	82 %	87 %	85 %	80.5
H5N1 : H9N2	87 %	93 %	90 %	63 %	94 %	60 %	92 %	73 %	81.5

## Table 1: Percentage homology of all the eight genes of H1N1, H2N2, H3N1, H5N1 and H9N2 calculated using Clustal W (1.82)

The nucleotide sequences of all segments of influenza-A virus subtypes similar regions were aligned by Clustal W. Based on the aligned regions of virus subtypes, it was found that segment-6 of H5N1 showed maximum similarity with H1N1, where as other segments (2, 3, 4, 5, 7 and 8) showed similarity with H9N2 (figure 2).

NC-004911. NC-007375. NC-002021. NC-007372. NC-007358.	50000 50000 50000
NC-004911. NC-007375. NC-002021. NC-007372. NC-007358.	100 100 100 100
NC:004911. NC:007375. NC:002021. NC:007372. NC:007358.	150 150 150 150 150
NC-004911. NC-007375. NC-002021. NC-007372. NC-007358.	NNNNN NNNNN 00000
NC:004911, NC:007375, NC:002021, NC:007372, NC:007358,	200 200 200 200 200 200 200 200 200 200
NC-004911, NC-007375, NC-002021, NC-002021, NC-007372, NC-007358,	3000 3000 3000 3000

a. Segment-2

NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.		50 50 50 50
NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.	CAN DECOMPT GAT TOTE CONSTRUCTION CONSTRUCTO CONSTRUCTICO	100 100 100 100
NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.	ССССААААТТ <u>САСАССАССАССТАТАСССССССССССС</u>	150 150 150 150
NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.	TOTROTICAL BETATICADARDITOCAL TICAL TRANSPACE ADDRAGO CAAACA AAAGTATIGDETICATGIATTICACATITITOATENATGAGCAAGGCCGA GAAGTATIGDETICATGIATTICACATITITOATENATGAGCAAGGCCGA GAAGTATIGDETICATGIATTICACATITITOATENICATCAATGAGCAAGGCCGA GAAGTATIGTENICATGIATTICAGATITITOATENICATCAATGAACGAGGCGA GAAGTATIGTENICATGIATTICAGATITITOATENICATCAATGAACGAGGCGA	200 200 200 200 200
NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.	СТАКЦЕСТВАЛСССВАТСССКАТ СССТО ПТОЛОСССВАТСССВА ССССВАТСССА ОПСААТААТССТАСАЩОТТСВЕ САТССЕКАТСССКТЕ ПТОСАХССАСАСАТ ОПСААТААТСЕТТСВЕ САТССЕКАТССАСТЕ ПТОСАСССССССАСАТ СПСААТААТСЕТТСВАТСССКАТССАСТЕ ПТОСАССССКАТС СПСААТААТСЕТТСВА СССТСВАТСССКАТССАСТЕ ПТОСАСССССАСАТ АТСААСТЕТТСВА СССТСВАТСССКАТССАСТЕ ПТОСАСТССВЕ АТСААСТЕТТСТА СААСТСССКАТССАСТЕ ПТОСАСТССВЕ СТСААТЕ АТСААСТССВАТСССКАТССАСТЕ ПТОСАСТССВЕ С С С С С С С С С С С С С С С С С С С	260 260 260 260
NC-004912. NC-007376. NC-002022. NC-007371. NC-007359.	AATKAULTBERNAGBARANDESEERABEREN BERUKEN BERUKEN GONTON DE GANALTARCA EFTEGAAATAATEREN BERUKEN GANGATEREN BERUKEN GONTON DE GANALTARTAR ETTOAAATAATEREN BERUKEN GANGATEREN BERUKEN GONTON DE TAAAAG ETTOAAATAATEREN GERUKEN GONTON DE GANALTARCEN GONTON DE TAAAAG ETTOAAATAAT AATEREN GERUKEN GERUKANTEREN DE GAAATEREN GONTON GONTON DE TAAAAG ETTOAAATAAT AATEREN BERUKEN GERUKANTEREN DE GAACAATEREN GONTON DE TAAAAG ETTOAAATAAT AATEREN BERUKEN DE GAACAATEREN DE GAACAGET BETER AAF	300 300 300 300 300

b. Segment-3

NC-007377. NC-004607. NC-002016. NC-007367. NC-007363.		55000
NC-007377. NC-004997. NC-002016. NC-007367. NC-007363.	COTACELTCICCCATCOCCATCAGGCCCCCCTCAAAGCCCGAGATCCCCCCCCCC	1 000 1 000 1 000 1 000
NC-007377. NC-004907. NC-002016. NC-007367. NC-007363.	CACAGACTICAAGATCTICTICCTCCTCCAGAAGAACACACACACACACACACACACACAC	1500
NC-007377. NC-004997. NC-002016. NC-007367. NC-007363.	<u>CAT COAAT COCTAAACA CAACA COATCOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTOTO</u>	2000 2000 2000 2000 2000 2000 2000
NC-007377. NC-004907. NC-002016. NC-007367. NC-007363.	TTTTGGGGATTTGGTATTGAGGGTGAGGGTGCCAAGTGAGCGAGGACTGCAG AAGGGDAGATTTGGGGTTCACGGTGCCAAGTGAGCGAGGAGCACTGCAG TTTTGGGGTTTGGTGCCCACGGTGCCCAGTGAGCGAGGAGCAGGAGCAGGAG TTTTGGGGTTTGGTGTCACGGCTCACCGGTGCCCAGTGAGCGAGGAACCGAGGAACTGCAG	260 260 250 250 250
NC-007377. NC-004907. NC-002016. NC-007367. NG-007363.	COT A CACCOLT TT GT CCAAAT COLCT CAAT COCAAT TO CCAAT COCAAT COCAAT A COCAAT A COCAAT A COCAAT A COCAAT COCAA	3000 3000 3000 3000

е.	Segment-6

NG-007382. NG-002018. NG-004908.	50 50
NC-007361. NC-007366.	50
NC-007382. NC-002018. NC-004909. NC-007361. NC-007368.	100 100 100 100
NC-007382. NC-002018. NC-004909. NC-007361. NC-007368.	150 150 150 150
NG-007382. NG-002018. NG-004909. NG-007361. NG-007368.	200 200 200 200 200 200
NG-007382. NG-002018. NG-004909. NG-007361. NG-007368.	250 250 250 250 250
NC-007382. NC-002018. NC-004909. NC-007361. NC-007368.	3000 33000 3000 3000

NC-007381. NC-002019. NC-004905. NC-007369. NC-007360.	N <u>U OGCAT CECAA DE CACCAA E E CIICUTAT SAACEBA TECAA E TCAT GA</u> A GCAAAA GCA BG GTAGATAA TCACT CAA T SAB TGACA TCAAAA TCAT GGC A GCAAAA GCA BG GTAGATAA TCACT CA CT GA B T GACA TCAAAA TCAT GGC A GCAAAA GCA BG GTAGATAA TCACT CA CT GA BT GACA TCAAAA TCAT GGC A GCAAAA GCA BG GTAGATAA TCACT CA CT GA GT GACA TCAAA TA TCAT GGC	50 50 50 50
NC-007381. NC-002019. NC-004905. NC-007369. NC-007360.	СВААЕВССАСКАТССА АССВИСТ САСАТСАСКОСАТССВИСССССА КСАТСАТТС СТСССААСССАААССВИСТТАКВААСАСАСАТСОВИССКОТ ВАГОВА ВААС ОТОСССААСССАААССВИССИТАТСААСАСАСТОВАТСВИТССА САСТ СТСССААСССАААССВИТСИТАТСААСАСАТССААААСТОВИТССКОА СТСССААСССАААССВИТСИТТАТСААСАСАТССААААСТОВИ СОССИСАСССАААССВИТСИТТАТСААСАСАТССААААСТОВИ СОССИСАСССАААССВИТСИТТАТСААСАСАТССААААСТОВИ СССВИСИ СТСИСАВОССАССАААССВИТСИТТАТСААСАСАТССААААСТОВИ СССВИСИИ СТСИСАВОССАССАААССВИТСИТТАТСААСАСАТССААААСТОВИ СССВИСА	100 100 100 100 100
NC-007381. NC-002019. NC-004905. NC-007369. NC-007360.	ATCGARTEGRACGATTCTARALCCARRTGTCRCCGAACHERAACTCARE RCCCAGAATGCCERCTGARAACCAGARCATCGETCCGGAAGAATGATTCGTTCGGA GCCAGAATGCERCTGARAATCAGARGACATCCERTCRGAAGAATGGTTGGGA GCCAGAATGCERCTGAGATCAGBCCATCTERTCRGAAGAATGGTTGGA GCCAGAATGCERCTGAGATCAGBCGACCATCCERTFCGAAGATGATCGETGGA GCCAGAATGCERCTGAGATCAGBCGACCATCCERTFCGAAGATGATCGETGGA	150 150 150 150
NG-007381. NG-002019. NG-004905. NG-007369. NG-007360.		200 200 200 200 200
NG-007381. NG-002019. NG-004905. NG-007369. NG-00736D.	CTCTCTOTTTTTOARCACABAAGOAATAACAATACTORAACAACAF COPA LGARDAGACAGOTTGATCCAAAAAGAGAATAGGTOCTCT ARAAGGAAGAGATGGTOCTCT CGAAGGACAGGTTGATCCAGAACAGCTTTAACAATAGGAGAATGGTOCTCT LGAAGGACAGGTTGATCCCAGAACAGCTTEACAATAGGAGAATGGTOCTCT LGAAGGACAGAGAGAATAGGTOCTCT	250 250 250 250 250 250
NC-007381. NC-002019. NC-004905. NC-007369. NC-007360.	ЭССРЕДАЛ ВСАГССТАХСАА А АСТОССАССАТАТАСАА СВОДАСА СТОСТИТТСА ВКААССАСТАХА А АСТОССТВСА ДА АСАТСССАСТО СЕВССИТТТСАТСААССАССАСТАССТВСА ДА АСАТСССАСТОСС СТОСТИТТСАТСААССАССАСТАСАТАССТВСААСААСАТСССАС СТОСТИТТСАТСАААССАССАСТАСАТАССТВСААСААСАТСССАС СТОСТИТТСАТСАААССАССАСТАСАТАССТВСААСААСАТСССАС СТОСТИТТСАТСАААССАССАСТАСАТАССТВСААСААСАТСССАС СТОСТИТТСАТСАААССАССАСТАСАТАССТВСААСААСАТСССАСТССС	300 300 300 300

c.	Segment-4	
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NC-00490B. NC-007366. NC-007362. NC-007374.	CONTRACTOR DE LA CONTRACTA CONT	50 50 50
NC-00490B, NC-007366, NC-007362, NC-007374,	A START A LA TRACTACIÓN CA CALANTAL CON LA CONTRACIÓN DE LA CALANTAL CONTRA LA STARTA DE LA CALANTAL CONTRACA A CALANTAL CONTRACIÓN DE LA CALANTAL LA STARTA DE LA CALANTAL CONTRACIÓN DE LA CALANTAL CONTRACIÓN DE LA CALANTAL LA STARTA DE LA CALANTAL CALANTAL CONTRACIÓN DE LA CALANTAL CONTRACIÓN DE LA CALANTAL CONTRACIÓN DE LA CALANTAL	1 00 1 00 1 00 1 00
NC-004908. NC-007366. NC-007362. NC-007374.		150 150 150
NC-00490B. NC-007366. NC-007362. NC-007374.	CALLOLLECTOTOLOCACATOCOLARACIÓN COLOCACACACACIÓN TATO TACIÓN CALACANTRECEDATOLECACATULES ACONTACIÓN COLOCACIÓN CALLOCACACACACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN CALLOCACACACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN COLOCACIÓN CALCONTROCACIÓN COLOCACIÓN COLO	200 200 200 200
NC-00490B. NG-007366. NC-007362. NC-007374.	SATISTICS CARCENES CONCENTS CO	250 260 250 250
NC-004908. NC-007366. NC-007362. NC-007374.	A FIALT BAAGGAA GEARD CITER COACAA CATTER FOR A CORRENT OF OG CEITER TONICA AA CITAA TAON TO CITATI CORCAN SA SECTA FICH REDATA COCTATOR SA CRATIFICATOR OF OCCORA TOGICOT INFRANCIA COCTATOR STOCK COACATOR CONTACT OF TA SECTION	300 300 300 300
NG-00490B. NG-007366. NG-007362. NG-007374.	A a company     A a	360 360 360





NC-00738D. NC-007364. NC-00202D. NC-00737D. NC-004908.	AN G <u>GARTICTAA CACTOTOTOTAA GINNITOTAABORAGATTOCINOCONNICAA</u> GIO <u>GEAA A GEA TAATOGARIICCAA CACCACTOTICAN DE ABORTAA CACTOTOTO A GE</u> A GCAAAA GEA GGOT <u>GA CAAAGA CATAA TGGA TOCAA CACTOTOTOTAA GE</u> A GCAAAA GEA GGOT <u>GA CAAAGA CATAA TGGA TICAA CACTOTOTOA A G</u> E A GCAAAA GEA GGOT <u>GA CAAAGA CATAA TGGA TICCAA CACTOTOTOA A</u> A GCAAAA GEA GGOT <u>GA CAAAGA CATAA TGGA TICCAA CACTOTOTOA G</u> E	50 50 50 50 50
NC-007380. NC-007364. NC-002020. NC-007370. NC-007370.	TORTOCHAARA GAARA TELEGINA GAACCAAGAA CIABGIGGI BOCCCCATTCCTTG THETA ARA GAACAGACCAAGACCCAAGACCCCATTCCTTGA THETA CAAGACTACAAGACCCAAGACCCCAGACAAGACAAGA	100 100 100 100
NC-00738D. NC-007364. NC-00202D. NC-00737D. NC-00737D. NG-004906.	AT COCCTTCCCCCCCCCTTCACACCTCCCTACCCCCACACCCCCACACCTCTC TECCECTCCCCCCCCCC	150 150 150 150
NG-00738D. NG-007364. NG-0022D. NG-00737D. NG-00737D. NG-004906.	COLTAACA BOGAA GA BOCA COLOCIC FEET OF BOACA T CABA OF A GA OA O CACOCCA ACADA COLTBOO CACOCCAC TO THE ACADOR OF COLTACA ACTOCCAC OF BOACA COLOCICAACABA OF ACTOCAC TO THE ACADOR OF COLTACA ACTOCCAC OF COLTACA ACADOR OF COLTACADOR OF COLTACA ACADO	200 200 200 200 200
NG-00738D. NG-007364. NG-00202D. NG-00737D. NG-00737D. NG-004906.		250 250 250 250 250
NC-00738D. NC-007364. NC-002020. NC-00737D. NC-00737D.		300 300 300 300 300
	a Commont O	

g. Segment-8

Figure 2: Multiple Sequence alignments of different segments i.e. seg-2 to seg- 8 (a to g) of subtypes (H2N2, H5N1, H1N1, H3N1 and H9N2) of influenza-A virus. In H5N1 all the segments shows maximum aligned regions with H9N2, except segment -6 of H5N1 and it shows more aligned regions with H1N1.

#### Phylogenetic Analysis of Segments of Subtypes of Influenza-A

The gene segment-2 of H5N1 shows close resemblance compared with segment -2 of H9N2.The influenza-A subtypes of H2N2 and H5N1 are closely related and the cluster of gene segments -2 of H1N1 and H9N2 is distantly related compared with H3N2 as shown in figure (3). The unrooted phylogenetic tree of segment 3 in the selected five subtypes of Influenza A virus presented in figure (4 & 5). There is no common ancestor for all the third segments and it clearly shows the close resemblance between H5N1 and H9N2. The phylogenetic tree of segment 4 in the selected five subtypes of Influenza A virus showed the close resemblance between H3N2 and H2N2 (figure 6 & 7). The gene segment 5 in the selected five subtypes of Influenza A virus shows the close likeness between H5N1 and H9N2in figure(8 & 9). Phylogenetic tree of segment 6 in the selected five subtypes of influenza-A virus reveals close semblance between H5N1 and H1N1. The cluster of these gene segments are distantly related to H9N2 as shown in figure(11). The phylogenetic tree analysis of segment 7 and segment-8 in the selected five subtypes of Influenza A virus shown the close resemblance between H5N1 and H9N2 (figures 12,13&14). The haemaglutinin of selected subtypes of influenza-A shows close resemblance between H5N1 and H1N2 (figure 15,16&17). Similarly, neuraminidase of subtypes of influenza-A reveals close relation between H5N1 and H1N1 (figures 18, 19 & 20).



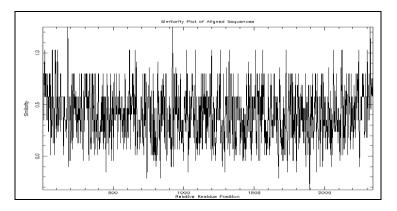


Figure 1: Similarity regions of nucleotides in segment 2 of H1N1, H2N2, H3N2, H5N1 and H9N2 by PLOTCON

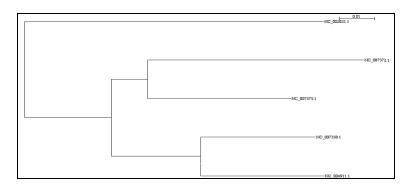
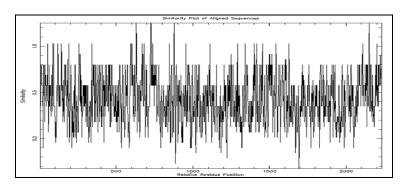
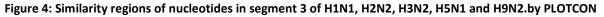


Figure 3: Phylogenetic tree of segment-2 of H1N1, H2N2, H3N2, H5N1 and H9N2





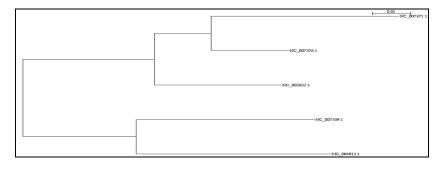


Figure 5: Phylogenetic tree of segment-3 of H1N1, H2N2, H3N2, H5N1 and H9N2



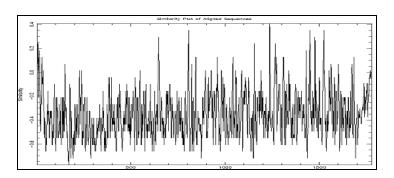


Figure 6: Similarity regions of nucleotides in segment 4 of H1N1, H2N2, H3N2, H5N1 and H9N2.by PLOTCON

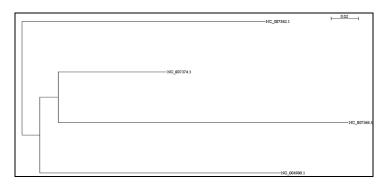


Figure 7: Phylogenetic tree of segment- 4 of H9N2, H3N2, H5N2, H2N2, and H2N2

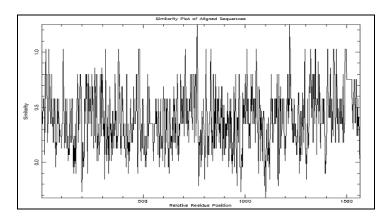


Figure 8: Similarity regions of nucleotides in segment 5 of H1N1, H2N2, H3N2, H5N1 and H9N2.by PLOTCON

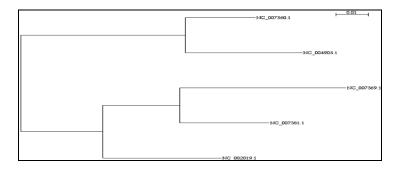


Figure 9: Phylogenetic tree of segment- 5 of H1N1, H2N2, H3N2, H5N1 and H9N2

May-June



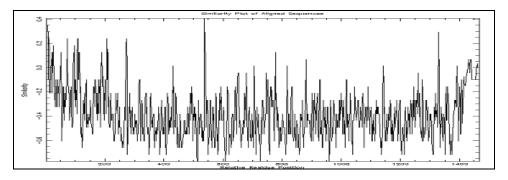


Figure 10: Similarity regions of nucleotides in segment 6 of H1N1, H2N2, H3N2, H5N1 and H9N2.by PLOTCON

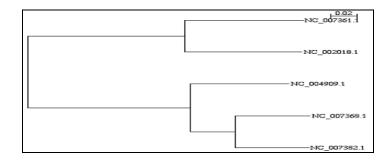
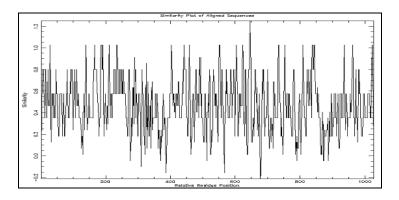


Figure 11: Phylogenetic tree of segment- 6 of H1N1, H2N2, H3N2, H5N1 and H9N2



#### Figure 12: Similarity regions of nucleotides in segment 7 of H1N1, H2N2, H3N2, H5N1 and H9N2.by PLOTCON

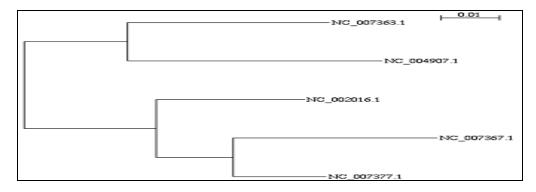


Figure 13: Phylogenetic tree of segment- 7 of H1N1, H2N2, H3N2, H5N1 and H9N2

May-June



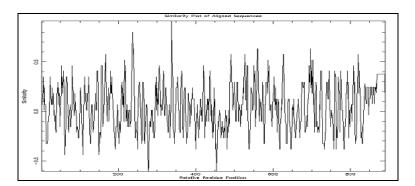
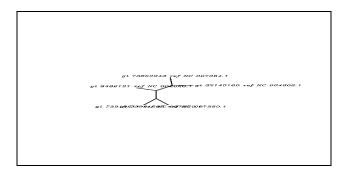
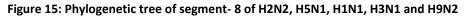


Figure 14: Similarity regions of nucleotides in segment- 8 of H2N2, H5N1, H1N1, H3N1 and H9N2 by PLOTCON





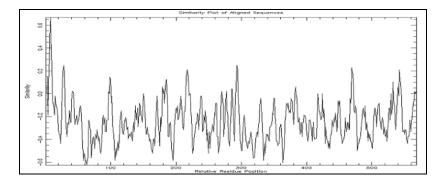


Figure 16: Similarity regions of aminoacids in Hemagglutinin of H1N1, H2N2 and H5N1

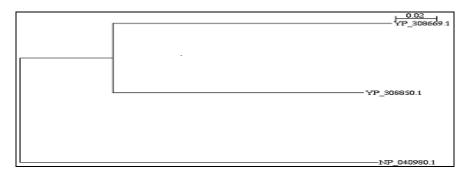


Figure 17: Phylogenetic tree of Hemagglutinin of H1N1, H2N2, and H5N1



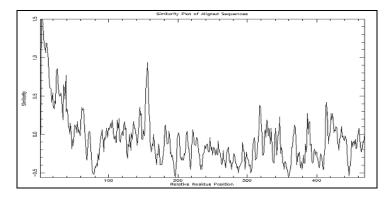


Figure 18: Similarity regions of amino acids in Neuraminidase of H5N1, H1N1, and H2N2

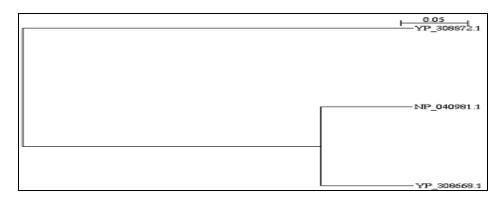


Figure 19: Phylogenetic tree of Neuraminidase of H5N1, H1N1, and H2N2

NP-040980. YP-308850. YP-308669.		50 50 50
NP-040980.	DE MOKLCRIK GLAPLOIGKONI AG WALGNPEDEP L PYRGESY Y YETP	100
YP-308850.	THREKLCKLNGG PLOIGKONI AG WALGNPECERLLS PROGESY THE END	100
YP-308669.	THREKLCKLNGG PLOIGY AG WALGNPECERLLS PROVE WAY Y L A S	100
NP-040980.	N E BOI SY PORT I TET LREQLORYSSE FEE FEE SMP HITTKOYT	150
YP-308850.	RYFECYROSFUT REFEITHLBERVKIFEENT I PKDEW ORT TGG WEDE	150
YP-308669.	PLOY PGD PUN PEEL KHLBER KHFET O DPKSSWENTER BY BE	150
NP-040980. YP-308850. YP-308669.	A CONTRACTOR OF A CONTRACTOR O	200 200 200
NP-040980.	NSDCONTYONE RAYESMETSNYNRRFTPEIAEDRYKTRDGAGRMNDYWTL	250
YP-308850.	ORALYDNYGATYVEVETSTRKRSIPEIAARPEYNGLGRRMEDFIN LDUW	250
YP-308669.	AEDTLYGNPTYVEVEDSDNDRLVPEIAFYDGLGRRMEDFDUI LV	250
NP-040980.	LKPGDTIIFEANDALIAPPYAFALBROFGSGIIBAASMHEONTECGTPL	300
YP-308850.	DTINFESIGNANYAFEYGFAFKGGKGGGAMITBELENGGETGGCTPLGAIN	300
YP-308669.	PNDAINFESINAYAFEYGFAFYKIVKKGGGAINMITGELENGGCTPLPMGA	300
NP-040980. YP-308850. YP-308669.	GA BSLPFOL HPTIGECPKYVRSAK KINTGLIN PSIBOR GA TTPFHVVRPLT CECOPKYVRSAK KINTGLIN PSIBOR GA TSBMPFHDIP COPKYVRS KLVK (TGRVP) BRECKA KOFF I NSBMPFHDIP COPCOPKYV BINKL DA FOLKNIT DARER FOR ACTED	350 350 350
NP-040980.	AGEI GRATENI DOM BEN BELEGGE GYA BEKET GNA I NG TUK NA MA	400
YP-308850.	EBRWERY DEN YOY HARNOGE BYA BELEFIGI I NAKI NG TUK NA	400
YP-308669.	El astronom vowngen is neders gya adat e to gai dogy ni vni	400



YP-308668.	MNPNOKIITIGSICMVVCIISLNLOIGNIISIWVSHSICTCNOHDEPPN	50
NP-040981.	MNPNOKIITIGSICLVVCLISLILOIGNIISIWISHSICTCSOHHTGIDN	50
YP-308872.	MNPNOKIITIGSICLVVCLISLILOIGNIISIWISHSICTCSOHHTGIDN	50
YP-308568.	QSIITYENNTWYNDTYYN SNENELTEKAYASYTLEGNSSICPESGWAYH	1 00
NP-040981.	QNIITYKNSTWYKDTESFILTENSSLOPIRGWAIYAKDNSIRIGBKGDYF	1 00
YP-308872.	MPCEPITTERN TEFYYN NTTFEKEICPEVYEYRNW ARPOCDIGFAPE	1 00
YP-308868.	SKDNGERTBSKGDVFV)REPFISCSALEORTFJEFTØGALENDKHSNBTVK	150
NP-040981.	VIREPFISCSHLEDRIFFLIGGELLIDAHSNGTVKDREPVRALMSDPVGE	150
YP-308872.	SKDNSERLSSGDIVVIREPIVSCOPSKCVQFALSSGTFLDNKHSNDFTH	160
YP-308668.	DRSPHRTLINGOPEOFAPEPENSEFESYAWEASACHDOTEWEITIGISGPEN	200
NP-040981.	APPPYNERFEYAWSABLCHINGWENTIGISBPDNGAVEVLYYNGIITEE	200
YP-308872.	DRIPHRTLINNEEGYPFHLGTRIVOLAWSESSCHDBKAWLHVOVTGDDEN	200
YP-308668.	GAV LYVNG TIDTIK SWENN D. RTEESEC CVNGEC FT∭NT BEPEN GE	250
NP-040981.	IKEWRKK I LRTGESEC CVNGEC FTIMT BEPEDGLASYK FKIEK KVTK	260
YP-308872.	AT SFLYDGRL™SJGWSDN DRDGESECVCINGTCTV DTDGSABGRA	260
YP-308568.	NSYK PKNEKORYVKSVELNOPNOHYEECOOPDAGEITOVORONNOON	300
NP-040981.	E E NAPNSHYLEOSOYPDIGKVMOVORDUHOSNRPWYSFDUNLDUOIG	300
YP-308872.	DRLF) EEGKIMHISPLSGAQOVEECSOPRYPDVRCICRONWKGSOR	300
YP-308668.	RPWYSFNDMLEYDIB <u>FICSOVFDDNPRPNDDCGCCGPVEPNGAY</u> BYKDFS	360
NP-040981.	YFCSGVFDDNPRPKDGTGBCGPVYVDGANBVKBFBYRYGNGVWIBRTKSH	360
YP-308872.	PVIDINMEDYSIDSS <u>VVCSG</u> VBDTPRNUDRESISNCRIPNNERDNPBYK	360
YP-308868.	FKYGUSYW ORTKSINS BGFEN WOPNGWIEIDSS SYKODIYA ITDWS	400
NP-040981.	SSRHOFEN WOPNGWIEIDS FSLRODVFAN TDWSSISGFFDHPET BL	400
YP-308872.	GWAFDNGDDYWMGRIDSSDESGYEIFKY GEWSIPNEKSON ROLVDS	400

Figure 20: Multiple Sequence alignments Hemagglutinin and Neuraminidase segments of H1N1, H2N2, and H5N1

#### ACKNOWLEDGEMENTS

The authors would like to thank Dr. L. Rathaiah, Chairman and Prof. M.S.C Bose, Vice Chancellor, Dr.S.Krupanidhi, HOD, School of Biotechnology, Vignan University for providing computing facilities to carry out this work.

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#### ISSN: 0975-8585



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