Characterization of genetic changes in influenza viruses H1N1pdm09 during 2015/2016 epidemic season in Ukraine

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Introduction

This year's seasonal influenza risk assessment identifies type A viruses, in particular A(H1N1) pdm09, as dominant thus far in EU/EEA countries. The A(H1N1)pdm09 virus is responsible for the hospitalization of a large number of severe cases and 391 human death in Ukraine.

Regardless of the long-term research of influenza variability the reasons of occurrence of new virus strains and spread patterns of epidemic causative agents still remain undetermined. Investigation of molecular and genetic variations in gene segments of the flu virus is the key to forecasting and understanding the occurrence of future epidemics. Thus **the purpose** of this paper is to determine genetic features of pandemic influenza viruses that have been circulating during the season 2015-2016 to forecast following possible molecular and genetic variations in viruses.

Methods

Samples were analyzed using real-time polymerase chain reaction (RT-PCR). The sequences of influenza viruses from other countries were received from web-site GISAID using BLAST analysis. Sequences were aligned using ClustalW algorithm. The influenza A(H1N1)pdm09 sequences are characterized in a neighbor-joining phylogenetic tree with reference strains rooted from the current vaccine strain, A/California/07/2009-like virus. Phylogenetic analysis was performed using MEGA 7. 3D structures were constructed in Chimera 1.11.2rc software.

Results

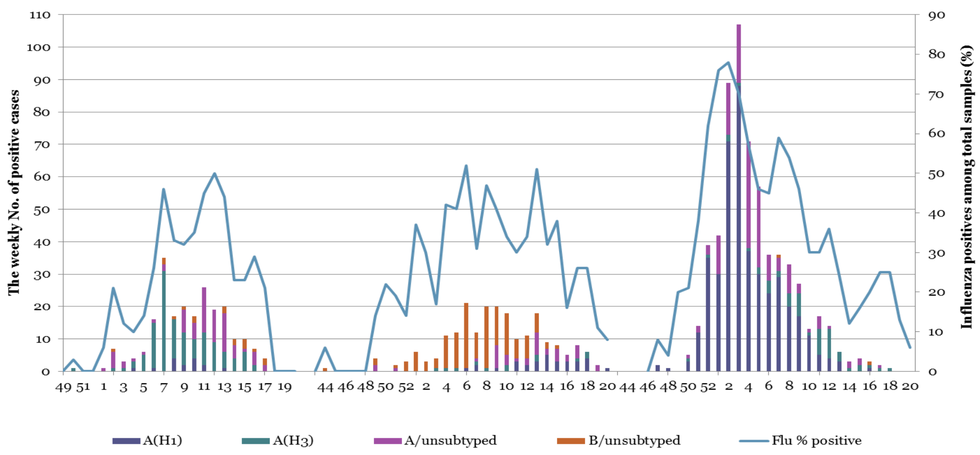

Fig.1. The weekly number of laboratory confirmed influenza cases and percent with the percentage influenza positive SARI cases in 2013/14, 2014/15 and 2015/16 influenza seasons in Ukraine

Genetic variations of pandemic influenza viruses were analyzed according to genes of surface proteins haemagglutinin (HA) and neuraminidase (NA), and non-structure protein (NS1).

Over the last five years the HA genes have evolved and eight genetic groups have been designated, with A/California/7/2009 representing group 1, and viruses in group 6 have formed clusters designated groups 6A, 6B and 6C. Viruses collected in 2015-2016 season fell into genetic group 6B and in two emerging subgroups, 6B.1 and 6B.2.

These subgroups are marked by specific amino acid substitutions. Most isolates were specific to the new genetic group 6B.1 that occurred in the middle of 2015. This group had specific mutations S84N, S162N and I216T. Four Ukrainian isolates from Odessa region were specific to 6B.2 subgroup and acquired amino acid substitutions R113K, D127E (acquisition of a potential glycosylation site). It is expected that the following epidemic seasons viral spread will be limited to 6B.1 genetic group.

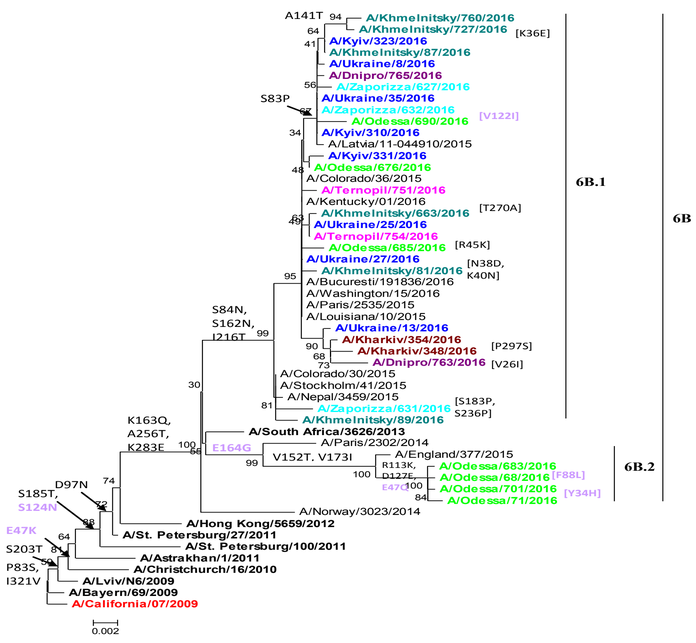

Fig.2. Phylogenetic analysis of the HA nucleotide sequences of influenza A(H1N1)pdm09 viruses

It is known that the H1 HA molecules have four distinct antigenic sites: Sa, Sb, Ca, and Cb. As a result, these sites consist of the most variable amino acids in the HA molecule of the seasonal human H1N1 viruses that have been subjected to antibody-mediated immune pressure. Notably, the Sa and Sb sites that contain many amino acids involved in neutralizing epitopes near the receptor binding pockets.

In Ukrainian isolates were observed mutations in antigenic sites, which emerged in 2015-2016 epidemic season. The main substitution S162N emerged in Sa antigenic site and was observed in all isolates from group 6B.1.

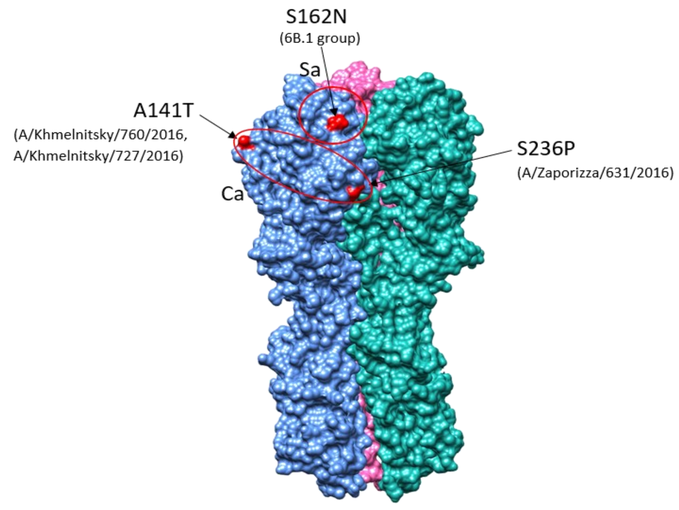

Fig.3. 3D structure of antigenic sites on the HA molecule of Ukrainian isolates.

Two substitutions were observed in antigenic site Ca, A141T – had isolates №727 and №760 from Khmelnitsky, S236P – in A/Zaporizza/631/2016. Information about changes in antigenic sites very important for prediction next dominant strains.

Genetic comparison of influenza virus A(H1N1)pdm neuraminidase genes shown that all investigated isolates were genetically related to reference strain A/South Africa/3626/2013 and saved high genetic similarity to vaccines strain A/California/07/2009. All discovered viruses retain susceptibility to oseltamivir and zanamivir.

Viral NS1 protein plays a central role in counteracting host cell processes that try to interfere with viral replication.. In 2015-2016 epidemic season in Ukrainian isolates amino acid substitutions D2E, N48S, and E125D were identified in the NS1 protein. These mutations were absent in isolates in 2014-2015 epidemic season. Substitutions D2E and E125D occurred in 70% Ukrainian viruses and N48S in 12,5% of sequenced viruses.
Ukrainian isolates 2015-2016 season have been divided into two groups.

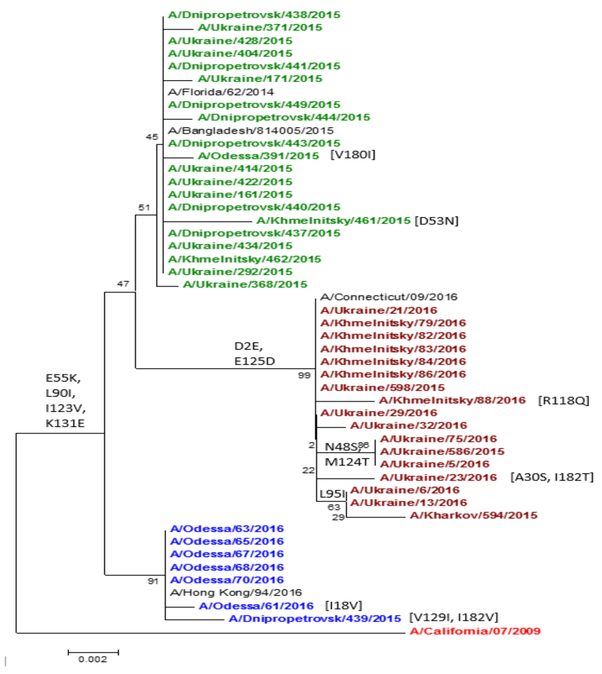

Fig.4. Phylogenetic analysis of the NS1 gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season.

An EpiFlu database search revealed that the frequency of substitutions D2E and E125D in NS1 protein of influenza A(H1N1)pdm09 viruses drastically increased in less than 1 year from 10% in 2015 in the Southern Hemisphere epidemic season to 74% in 2015/2016 in the Northern Hemisphere epidemic season .

Conclusions.

Genetic analysis of influenza A(H1N1)pdm09 viruses circulating in Ukraine in the 2015/2016 epidemic season showed that all of them were similar to the vaccine strain recommended by WHO. Viruses had acquired amino acid substitutions in HA molecule antigenic sites, which can lead to antigenic changes at the next epidemic seasons. Although new genetic subgroups have emerged in 2015-2016 epidemic season, the A (H1N1)pdm09 viruses received were antigenically similar to the vaccine virus A/California/7/2009 and retain susceptibility to oseltamivir and zanamivir.

Detailed analysis of substitutions in the protein encoded by internal gene NS1 showed that most of Ukrainian viruses acquired specific amino acid changes: D2E, N48S and E125D. E125D in NS1 is known to be one of the key substitutions involved in shutdown of host mRNA transport, restoring inherent disability of A(H1N1)pdm09 virus to efficiently control human cell gene expression.

The observed rapid spread of influenza A(H1N1)pdm09 viruses with no significant antigenic changes in HA can be speculatively explained by increased transmissibility, as well as by increased virulence or by combination of both. The possible link between transmissibility or virulence and described changes in NS1 internal gene in influenza A(H1N1) pdm09 viruses awaits experimental proof.