AN DIGENIC AND GENETIC ANALYSIS OF PANDEMIC INFLUENZA A(H1N1)2009 VIRUSES FROM PORTUGAL

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Background:
In April 2009, a new pandemic strain of influenza infected thousands of persons in Mexico and the United States and spread rapidly worldwide. This virus had origin in a reassortment between a North American swine lineage (already a triple reassortant, circulating in pigs since the late 1990’s) and a Eurasian swine lineage (Garten et al., 2009). The influenza pandemic virus (A(H1N1) 2009) was first detected in Portugal in 4th May 2009. The early diversification of the A(H1N1) 2009 viruses (based on concatenated whole genomes) resulted into seven lineages, clade 1-7 (Nelson et al., 2009). However, none of the amino acid changes that define clades are located in HA antigenic sites or is associated in the NA with resistance to antiviral drugs (Nelson et al., 2009).

In contrast with recent seasonal human H1N1 viruses, the A(H1N1) 2009 hemagglutinin (North American swine lineage) contains almost the same amino acid composition in the antigenic sites that pandemic 1918 H1N1 influenza viruses (Igarashi et al., 2010). As A(H1N1) 2009 viruses continue to circulate in the human population, HA antigenic sites will continue to be targeted by antibody-mediated selection pressure and certainly acquire amino acid substitutions. Therefore it is important, also from a public health perspective, continue to characterize the HA and to monitoring the antigenic and genetic properties of the H1N1 pandemic viruses in order to detect any changes and thus any necessity for selecting further vaccine candidates or changes in antiviral recommendations.

In this study, is presented a genetic and antigenic characterization of influenza A(H1N1) 2009 viruses, isolated in Portugal over the 2009 influenza pandemic.

Material and Methods:
In Portugal, during the 2009 influenza pandemic, about 16500 clinical samples were tested by the National Influenza Reference Laboratory for the presence of influenza A(H1N1) 2009 virus. From near 8000 A(H1N1) 2009-positive (real-time RT-PCR) samples, 147 were isolated in MDCK-SAT1 cell cultures and characterized antigenically by hemagglutination-inhibition assays (HI) performed by the WHOCC - London. Of these, 56 isolates were taken for sequence analysis of the HA1 gene segment. Nucleotide sequences were aligned (ClustalW) and the phylogenetic trees constructed (Neighbour-joining, Kimura 2 parameter) using MEGA 4.0 software.

Results:
Antigenically, A(H1N1) 2009 Portuguese strains are homogeneous, being similar to A/California/7/2009 and the later pandemic H1N1 viruses A/Bayern/69/2009 and A/Liv/N6/2009 (Table I). However, 12 isolated strains show 4-fold or greater titre reductions against most of the HI sera panel. They react better with A/Bayern/69/2009 and A/Liv/N6/2009 antisera (Table I). Three of these viral isolates present amino acid substitutions at HA1 antigenic sites: G155E (epitope B) in A/Lisboa/120/2009, R205K (epitope D) in A/Lisboa/73/2009 and E258D (epitope D) in A/Lisboa/140/2009. Was also observed the amino acid substitution V199I (vicinity of epitope D) in A/Lisboa/57/2009 hemagglutinin (Table II).

Sequenced hemagglutinins of Portuguese isolates, with two exceptions, belong to the clade 7, already described in the literature (Figure 1). As known, viruses from this clade have a S203T mutation in the HA and V190I and N285D in the NA, which are also found in clades 5 and 6 (Table II and Figure 1). One of our strains, A/Lisboa/31/2009, belongs to clade 6, as presents the Q203H amino acid change. This viral strain was isolated from a patient that arrived from the USA (Boston, New York) in June 2009. Another viral strain, A/Lisboa/35/2009, belongs to an earlier clade (at least, previous to clade 4) because it doesn’t presents the S203T neither the Q203H in its hemagglutinin and it lacks also the V190I and N285D amino acid changes in its neuraminidase (this patient arrived from Spain). Mutations G121V (A/Lisboa/75/2009) and P153L (A/Lisboa/159/2009 and A/Lisboa/160/2009) were located in the antigenic site G: G155E (A/Lisboa/120/2009 was found in site B; G170R (A/Lisboa/65/2009), R205K (A/Lisboa/73/2009) and D232E (observed in 23 isolated viruses) were located in site D. ET83 (A/Lisboa/61/2009), S74G (A/Lisboa/71/2009), P58S (all Portuguese isolates), M257I (A/Lisboa/159/2009 and A/Lisboa/157/2009) and E258D (A/Lisboa/140/2009) were located in antigenic site E (Table II). Further, mutations Q39W (A/Lisboa/83/2009) and Q293H (A/Lisboa/31/2010) were found to be in the vicinity of site C and V199 (A/Lisboa/35/2009) and S203T (observed in 54 isolated viruses) were found to be in the vicinity of site D respectively (Table II).

Conclusions:
The great majority of influenza A H1N1 2009 viruses isolated in Portugal were similar to the vaccine strain A/California/7/2009. They were mostly representative of the clade 7, except two strains with foreign travel history. Most of the observed amino acid changes in the NA were located at antigenic sites or in their vicinity. However, changes in positions 153-157 of the HA have been highly associated with reduced HI titers with ferret antisera to the A/California/7/2009 vaccine virus and usually emerge after virus propagation in cell cultures (Yang et al., 2010). This was observed in one “low-reactor” strain, A/Lisboa/120/2009, which carried the G155E substitution in epitope B. The permanent and global monitoring of the antigenic and genetic characteristics of A(H1N1) 2009 is essential to understand the evolution of these viruses, in order to select new vaccine virus candidates.

Bibliography:

Figure 1 – Phylogenetic tree of HA1 nucleotide sequences from pandemic influenza A(H1N1)2009 strains isolated in Portugal (Neighbour-joining method, Kimura 2 parameter model, bootstrap values above 50 are shown — 2000 replications). Reference strains in red.