Molecular characterization of influenza A viruses circulating in Cuba between April 2009 and August 2010


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Mutations that affect any of the eight gene segments of influenza A viruses, especially the HA segment (and its HA1 region), may induce immune evasion by reshaping antigenic epitopes. Since molecular markers of human host adaptation were not found for the A(H1N1)pdm09 viruses, unrecognized molecular determinants might have been associated with transmission to humans [5]. The evolution of circulating viruses is monitored via systematic molecular surveillance, which allows for detecting emerging genetic variants and their genetic match with the vaccine strain.

Given these points, the aim of the present study was the molecular and phylogenetic analysis of influenza A viruses circulating in Cuba between April 2009 and August 2010.

Between April 2009 and August 2010, nasopharyngeal swabs, bronchoalveolar fluids and lung tissue samples were sent from the all over the country to the National Influenza Center (NIC), located at the Influenza Virus Laboratory, Pedro Kouri Institute of Tropical Medicine (IPK), for diagnosis and surveillance purposes. All clinical samples were accompanied by a standard questionnaire containing epidemiological and clinical data, based on ethics considerations and with informed consent previously signed by each patient. Samples included in the present study were randomly selected from the total of specimens received at the NIC and they were representative for all periods (beginning, middle and the end of the epidemic season). Twenty-three clinical samples positive to influenza A viruses, four to the influenza A(H1N1), eight to the influenza A(H3N2),
and eleven to the influenza A(H1N1)pdm09, were included in this study. For each sample, the nucleotide sequence was obtained and compared to those from the vaccine strains recommended by the WHO for the 2009–2010 Northern Hemisphere influenza season [6], such as A/Perth/16/2009 (H3N2), A/Brisbane/59/2007 (H1N1), and A/California/07/2009 (H1N1)pdm09.

Total viral RNA was extracted from clinical samples using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, USA). Amplification reactions were performed according to a previously published protocol [7]. Amplicons were sequenced using the BigDye Genome Lab Dye Terminator Cycle Sequencing Kit (Beckman Coulter, Brea, USA). MEGA v.5.05 by means of the Kimura two-parameter model was used to calculate genetic distances. Phylogenetic trees were obtained with MrBayes v.3.1.2 software [8], and were visualized with Dendroscope v.2.7.4. Potential N-glycosylation sites for the influenza A(H1N1)pdm09 were predicted through NetNGlyc server 1.0.

The sequences reported in this study can be located in the GenBank database under the following accession numbers: JX853783; JX853785; JX853786–JX853787; KC914966–KC914967; KC934965; JQ922291; JQ922292; JQ922294–JQ922297; JQ922273; JQ922275; JQ922277; JQ922286; JQ922286; JQ922288; and KC914933–KC914936.

All the HA sequences corresponding to the A(H3N2) subtype were closely related to the A/Perth/16/2009 lineage (Figure 1a), with similarities of 97.84% and 98.21% for nucleotide and amino acid sequences, respectively. In particular, amino acid sequences were characterized by the presence of substitutions S241I and T212A in antigenic site D, and K144N in the antigenic site A. Nevertheless, Falchi et al.[9] reported amino acid variations for the antigenic sites A, B, D and E of the influenza A(H3N2) viruses. Additionally, Dapat et al.[10] detected five mutations in the HA gene (domain HA1) affecting the antigenic sites B, C, D and E. Suwannakarn et al. reported similar findings [11]. For the A(H3N2) influenza viruses, amino acid substitutions clustered generally in the antigenic sites A, B, D and E.

The sequences corresponding to the A(H1N1) subtype were closely associated with the A/Brisbane/59/2007 lineage (Figure 1b). The homology with the A/Brisbane/59/2007 lineage was 97.22% and 98.45% for nucleotide and amino acid sequences, respectively.
Substitutions S145N in the antigenic site Ca, and A193T, G189S and G189A in the antigenic site Sb, were detected. Suwannakarn et al.[11] found amino acid variations for the A(H1N1) influenza viruses in the antigenic sites Sb, Ca and Cb. These findings indicate that the amino acid substitutions affecting the antigenic regions of the HA were more frequent for influenza A(H3N2) than for seasonal influenza A(H1N1) viruses. Interestingly, the difference in mutation rates may be explained by the evolutionary dynamics of these viruses. For the immunization with an influenza vaccine to be effective, frequent replacements of the A(H3N2) vaccine strain are required, as the A(H3N2) subtype exhibits a higher rate of antigenic variations when compared with the A (H1N1) subtype [11].

Cuban sequences corresponding to the A(H1N1)pdm09 subtype were closely related to the A/California/07/2009 lineage (Figure 1c), with similarities of 97.25% and 98.21% for nucleotide and amino acid sequences, respectively. In addition to mutations found for the A(H1N1) subtype, mutations N441I and V272I were detected. Substitution D222E was also identified in one sequence. With an unknown biological function, D222E is a low-frequency mutation that has been detected in other countries. Mutation D222G, detected also in other countries and associated with disease severity, was not detected for the A(H1N1)pdm09 influenza viruses in Cuba. This finding may explain the low mortality rate observed in Cuba. Furthermore, the phylogenetic analysis indicated that all Cuban sequences belonged to the clade 7 (posterior probability > 0.90).This clade, which is characterized by S203T mutation, was responsible for most of the pandemic burden worldwide [12]. Whether the predominance of S203T mutation in the antigenic site Ca stems from immune selection, adaptation to human hosts, or fitness optimization remains to be determined [13]. The S162N mutation in the antigenic site Sa, represented the acquisition of a N-glycosylation site.

In agreement with other studies, we confirm the genetic variability of the A (H3N2), (H1N1) and (H1N1)pdm09 influenza viruses. Moreover, our findings emphasize the significance of systematic molecular surveillance for the effective management of influenza epidemics and pandemics. The genetic characterization of influenza strains circulating in Cuba has contributed not only to complete the molecular variability profiles for influenza viruses in Latin American and the Caribbean region, but also to support the WHO Global Influenza Surveillance Program. Our phylogenetic analyses, which can be compared with other phylogenetic analysis worldwide, constitute a starting point for future research on the genetic variability of influenza viruses.

References


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