

Molecular Characterization and Phylogenetic Analysis of the Hemagglutinin 1 Protein of Human Influenza A Virus Subtype H1N1 Circulating in Kenya During 2007–2008

Abstract:

Background. Among influenza viruses, type A viruses exhibit the greatest genetic diversity, infect the widest range of host species, and cause the vast majority of cases of severe disease in humans, including cases during the great pandemics. The hemagglutinin 1 (HA1) domain of the HA protein contains the highest concentration of epitopes and, correspondingly, experiences the most intense positive selection pressure. Objectives. We sought to isolate and genetically characterize influenza A virus subtype H1N1 (A[H1N1]) circulating in Kenya during 2007–2008, using the HA1 protein. Methods. Nasopharyngeal swab specimens were collected from patients aged ≥ 2 months who presented to 8 healthcare facilities in Kenya with influenza-like illness. We tested specimens for seasonal influenza A viruses, using real-time reverse-transcription polymerase chain reaction (RT-PCR). Viruses were subtyped using subtype-specific primers. Specimens positive for seasonal A(H1N1) were inoculated onto Madin-Darby canine kidney cells for virus isolation. Viral RNAs were extracted from isolates, and the HA1 gene was amplified by RT-PCR, followed by nucleotide sequencing. Nucleotide sequences were assembled using BioEdit and translated into amino acid codes, using DS Gene, version 1.5. Multiple sequence alignments were performed using MUSCLE, version 3.6, and phylogenetic analysis was performed using MrBayes software. Results. We found that, similar to A/Brisbane/59/2007 (H1N1)-like virus, which was included in the southern hemisphere vaccine for the 2009 influenza season, all 2007 Kenyan viruses had D39N, R77K, T132V, K149R, and E277K amino acid substitutions, compared with A/Solomon Islands/3/2006 (H1N1)-like virus, a component of the southern hemisphere vaccine for the 2008 influenza season. However, the majority of 2008 viruses from Kenya also had R192K and R226Q substitutions, compared with A/Solomon Islands/3/2006 (H1N1)-like virus. These 2 changes occurred at the receptor binding site. The majority of the 2008 Kenyan isolates contained N187S, G189N, and A193T mutations, which differed from A/Brisbane/59/2007 (H1N1)-like virus. The A193T substitution is involved in binding the sialic acid receptor. Phylogenetically, the 2008 Kenyan isolates grouped into 2 clusters. The main cluster contained viruses with N187S and A193T changes; residue 187 is involved in receptor binding, whereas residue 193 is at antigenic site Sb. Conclusion. Overall, the major genetic variations that occurred in seasonal A(H1) viruses either affected receptor binding or altered epitopes at the immunodominant sites. These genetic variations in seasonal A(H1N1) isolated in Kenya during 2007–2008 highlight the importance of continuing surveillance and characterization of emerging drift variants of influenza virus in Africa.