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Genetic characterization of influenza viruses from influenza-related hospital admissions in the St. Petersburg and Valencia sites of the Global Influenza Hospital Surveillance Network during the 2013/14 influenza season

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ABSTRACT

Background: Continuous surveillance for genetic changes in circulating influenza viruses is needed to guide influenza prevention and control.
Objectives: To compare intra-seasonal influenza genetic diversity of hemagglutinin in influenza A strains isolated from influenza hospital admissions collected at two distinct sites during the same season.
Study design: Comparative phylogenetic analysis of full-length hemagglutinin genes from 77 isolated influenza A viruses from the St. Petersburg, Russian Federation and Valencia, Spain sites of the Global Influenza Hospital Surveillance Network (GIHSN) during the 2013/14 season.
Results: We found significant variability in A(H3N2) and A(H1N1)pdm09 viruses between the two sites, with nucleotide variation at antigenic positions much lower for A(H1N1)pdm09 than for A(H3N2) viruses. For A(H1N1)pdm09, antigenic sites differed by three to four amino acids from the vaccine strain, two of them common to all tested isolates. For A(H3N2) viruses, antigenic sites differed by six to nine amino acids from the vaccine strain, four of them common to all tested isolates. A fifth amino acid substitution in the antigenic sites of A(H3N2) defined a new clade, 3C.2. For both influenza A subtypes, pairwise amino acid distances between circulating viruses and vaccine strains were significantly higher at antigenic than at non-antigenic sites. Whereas A(H1N1)pdm09 viruses clustered with clade 6B and 94% of A(H3N2) with clade 3C.3, at both study sites A(H3N2) clade 3C.2 viruses emerged towards the end of the season, showing greater pairwise amino acid distances from the vaccine strain compared to the predominant clade 3C.3.
Conclusions: Influenza A antigenic variants differed between St. Petersburg and Valencia, and A(H3N2) clade 3C.2 viruses were characterized by more amino acid differences from the vaccine strain, especially at the antigenic sites.

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Abbreviations: aHap, haplotypes spanning either the antigenic sites; GIHSN, Global Influenza Hospital Surveillance Network; HA, hemagglutinin; Hap, full length HA gene haplotypes; k, the average number of pairwise differences; π, the average number of pairwise differences per site (or nucleotide diversity); RT-PCR, reverse-transcription polymerase chain reaction; S, number of segregating sites; θ, the heterozygosity per site expected in a population in mutation-drift equilibrium given the observed S value.

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1. Background

As a consequence of error-prone replication of the viral RNA genome and host immune pressure, the antibody-binding sites of influenza A hemagglutinins (HAs) continuously accumulate mutations [1,2]. The emergence of variants escaping natural or vaccine-induced immune responses (antigenic drift) leads to annual influenza epidemics in humans [3,4], a major cause of morbidity and mortality and a significant economic burden on health services [5]. Continuous surveillance of circulating influenza strains is needed to identify new mutations in HA that can escape from host antibody responses [6–8] and thereby guide influenza prevention and control [9].

2. Objectives

Here, we compared the intra-seasonal influenza HA genetic diversity and differences from the vaccine strains in the A(H3N2) and A(H1N1)pdm09 influenza viruses collected at two sites (Valencia, Spain and St. Petersburg, Russian Federation) of the Global Influenza Hospital Surveillance Network (GIHSN) during the 2013/14 Northern Hemisphere influenza season. The GIHSN focuses exclusively on severe cases of respiratory infection requiring hospitalization and conducts a prospective, active surveillance, hospital-based epidemiological study over consecutive seasons [10].

3. Study design

3.1. Samples

Clinical samples for the study were collected from a prospective surveillance scheme conducted at St. Petersburg, Russian Federation and Valencia, Spain during the 2013/14 Northern Hemisphere influenza season. Study details and inclusion criteria are described elsewhere [10]. A nasopharyngeal and a pharyngeal swab were obtained from each patient using flocked swabs, submerged in transport media, and frozen at −20 °C until shipment to the reference virology laboratory for analysis (Virology Laboratory, FISABIO-Public Health, Valencia, Spain and World Health Organization National Influenza Centre (RII NIC), St. Petersburg, Russian Federation).

3.2. Molecular diagnosis, typing, and sequencing of influenza viruses

Molecular diagnosis, typing, and sequencing of influenza viruses complied with the World Health Organization recommendations [11]. In Valencia, total nucleic acid was extracted using an automated method (Nuclisens Easy-Mag, bioMérieux, Lyon, France). Influenza A and B viruses were screened by multiplex real-time reverse-transcription polymerase chain reaction (RT-PCR) in a Lightcycler 480II (Roche Applied Science, Penzberg, Germany) as described [12,13]. A typing real-time RT-PCR assay was performed following the World Health Organization protocol [11] for all influenza A-positive samples.

In St. Petersburg, viral RNA was extracted using a RIBO-prep kit (InterLabService, Russian Federation) or an RNeasy Mini kit (Qiagen, Hilden, Germany). RT-PCR for influenza A and B viruses was performed using AmpliSens Influenza virus A/B-FL, AmpliSens Influenza virus H1/swine-FL, and AmpliSens Influenza virus A type-FL kits (InterLabService, Russian Federation) with reverse transcription using an AmpliSens Reverta-L kit (InterLab-Service, Russian Federation) or an AgPath-ID™ One-Step RT-PCR kit (Ambion, USA) with US Centers for Disease Control and Prevention primers and probes to determine the lineage (Yamagata or Victoria) for influenza B viruses.

HA nucleotide sequencing was performed on every fourth sample positive for influenza A (Valencia) or on convenience samples (cycle threshold values <25) taken at the beginning, middle, and end of the influenza season (St. Petersburg). At both sites, the complete HA gene (HA1/HA2 regions) was amplified by long-range RT-PCR and sequenced by standard Sanger chemistry using gene-specific primers for the corresponding virus subtype [11].

3.3. Influenza variability and phylogenetic analysis

3.3.1. Sequence data

Nucleotide alignments were obtained using the ClustalW algorithm integrated in BioEdit ver.7.2.5 (Ibis Biosciences, Carlsbad, CA, USA) [14], including reference sequences (defining known phylogenetic clades) from the Global Initiative on Sharing Avian Influenza Data (GISAID) database (Annex 1 of Supplementary information). Maximum-likelihood phylogenetic trees were reconstructed using the whole HA gene sequence with the online PhyML platform (http://www.atcg-montpellier.fr/phyml). The best-fitting nucleotide substitution model (general time-reversible + gamma distribution among the sites) was estimated using Modeltest [15] integrated in MEGA 5.0 [16]. Branch reliability was evaluated by an approximate likelihood-ratio test (Chi-squared statistic-based) with an interior branch cut-off value of 0.9. The trees were rooted on the 2013/14 recommended vaccine strains A/California/7/2009 for A(H1N1)pdm09 or A/Texas/50/2012 for A(H3N2) viruses [17].

3.3.2. Genetic complexity and diversity

Genetic polymorphism and diversity [18] between Valencia and St. Petersburg viruses were estimated with DnaSP version 5 [19]. All parameters were calculated for the whole HA gene region and for the HA1 and HA2 subunits separately, as well as for non-antigenic sites only or for antigenic sites only (A–E of the H3 scheme and their equivalents Sa, Sb, Ca1, Ca2 and Cb for H1; reviewed in [20]). Nucleotide diversity (haplotypes) was calculated either considering only antigenic sites (aHap) or considering the full-length HA gene (Hap), to estimate the fraction of individual viral isolates for each haplotype. Pairwise amino acid distances between individual viral isolates and the corresponding vaccine strain were calculated using the p-distance model implemented in MEGA 6 [21]. Non-parametric Kruskal–Wallis tests were used to compare pairwise amino acid distances from vaccine strains. All statistical analyses were performed using Stata version 12 (StataCorp, College Station, TX).

4. Results

4.1. Samples

Samples were collected from early December, 2013 to mid-March, 2014, and half were collected during the seasonal peak of activity (January, 2014 for A(H1N1)pdm09 and February, 2014 for A(H3N2) viruses). Complete HA gene sequences could be determined for 34 A(H1N1)pdm09 and 26 A(H3N2) isolates collected in Valencia and for eight A(H1N1)pdm09 and nine A(H3N2) isolates collected in St. Petersburg. For A(H1N1)pdm09-positive samples, patient ages ranged from 0.1 to 83 years, with a median of 62 years for Valencia and 5 years for St. Petersburg. For A(H3N2)-positive samples, patient ages ranged from 2 to 94 years, with a median of 73 years for Valencia and 18 years for St. Petersburg.

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