

Two years of experience in hospital surveillance for the severe influenza like illnesses in St. Petersburg: etiology, clinical characterization of diseases, antigenic and genetic properties of isolated influenza viruses

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ABSTRACT

In this paper, we analyze the etiology of the diseases occurring during two consecutive influenza epidemic seasons in St. Petersburg, Russian Federation. The analysis is based on the results of the PCR diagnostics of the clinical samples collected from patients hospitalized in three St. Petersburg hospitals with influenza like illnesses (ILI). It was shown that the influenza virus A(H1N1)pdm09 was the dominant causative agent during the 2012-2013 epidemic season while, in the 2013-2014 season, A(H3N2) virus was predominant among adults and children. The influenza B virus activity was high in the 2012-2013 season and low in the 2013-2014 season. During both seasons, the main causative agent for the hospitalization of young children was respiratory syncytial virus (RSV), followed by rhinovirus and influenza virus. The rate of involvement of parainfluenza, adenovirus, metapneumovirus and coronavirus was low and was negligible for bocavirus. Children 0-2 and 3-6 years old formed the group of patients that was affected by acute respiratory infection agents the most. Children younger than 3 months old were the major group of the intensive care unit (ICUs) patients and only 27.5% of them were adults. RSV and rhinovirus were the leading cause of ILI among the children admitted to ICU. Among the adult patients admitted to the ICU, only influenza A(H1N1)pdm09, A(H3N2) and B viruses were detected during both influenza seasons.

According to the results of the antigenic and genetic analysis, most influenza A(H1N1)pdm09 and A(H3N2) viruses circulating in St. Petersburg matched the vaccine strains recommended by the WHO for vaccine composition in the 2012-2013 and 2013-2014 seasons.

INTRODUCTION

Hospital-based surveillance for the influenza like illnesses (ILI) and the severe acute respiratory illnesses (SARI) is an important part of multiplex influenza surveillance that is currently underdeveloped. Early recognition of a pandemic caused by a novel unidentified virus, through the investigation of ILI and SARI, is important to start the timely implementation of the Pandemic Preparedness Plan measures and rapid response of National Healthcare Service.

The last pandemic events were caused by the novel swine origin influenza A(H1N1)pdm09 virus that spread initially from Mexico to the United States and Canada and then globally starting from the spring - fall 2009 and finishing in mid-February 2010. Rates of hospitalization and death during this pandemic varied widely between countries [1]. The share of the hospitalized patients admitted to intensive care units (ICUs) varied from 9% to 31% and the rate of death among them - from 14% to 46% [2-6]. Primary influenza pneumonia had a high mortality rate during pandemic among the immunocompromised

individuals and the patients with underlying co-morbid conditions as well as among the young, healthy adults [7].

This study had several objectives: i. to analyze the clinical data for ILI related to influenza and other respiratory viruses among hospitalized patients (hILI) in the 2012-2013 and 2013-2014 seasons; ii. to investigate the dependencies between the patients' age and the clinical characteristics of illnesses caused by influenza A(H1N1)pdm09, A(H3N2), and B viruses as detected by real-time reverse transcription (RT)-PCR analysis; iii. to compare these results with the conclusions from studies of diseases caused by other respiratory agents; iv. and finally to perform the antigenic and genetic analysis of the viruses isolated from hILI patients.

MATERIALS AND METHODS

Standard operating procedures for on-site activities in a global influenza hospital-based surveillance network (GIHSN) were used across all the clinical investigations

[8, 9]. The surveillance group of healthcare professionals, trained to follow a common reference protocol, collected data on hospitalized patients with diagnoses potentially associated with influenza, according to European Influenza Surveillance Network (EISN) case definition [10].

The Local Research Ethics Committee of the Research Institute of Influenza approved the GISHN study. All information included in this study was collected from patients who provided written informed consent in accordance with Local Research Ethics Committee requirements personally or through their legal representatives.

Study population

Non-institutionalized patients hospitalized for at least 24 h with a diagnosis possibly associated with influenza were considered eligible to be included in the study. Patients who were 5 or more years old had to meet the European Centre for Disease Prevention and Control's clinical case definition of ILI [10] for at least one of four systemic symptoms (fever or feverishness, headache, myalgia, or malaise) and at least one of three respiratory symptoms (cough, sore throat or shortness of breathing). Children younger than 5 years old, as well as other patients, should be admitted to the hospital within 7 days of the appearance of the indicated above symptoms potentially associated with influenza.

The study was conducted in three hospitals. Each of them had their own specific code: hospital code #1 with one ward for adults aged ≥ 17 years (60 beds); hospital code #2 with two wards for children aged from 0 to 17 years (60 beds each); hospital code # 3 with two wards for children aged from 0 to 17 years (60 beds each).

Hospitalization of patients with ILI was carried out according to the directions of physicians in different polyclinics or emergency services regardless of the patient residence area in St. Petersburg.

Data and sample collection

The patient's consent for participation in the study was obligatory. Nasopharyngeal swabs were taken from all of the patients. Additional pharyngeal swabs were collected from patients ≥ 14 years old and nasal samples were collected from younger patients. Samples were collected within 48 h of hospital admission and stored at -70°C before testing in the coordinating site's laboratory. The patient's status was recorded according to the answers to the core questionnaire that were given by the patient or his/her legal representative in the course of the face-to-face interview with the attending physicians.

Confirmation of influenza infection

Real-time multiplex reverse transcription (RT)-PCR was performed according to the procedures described in the manufacturer's instructions (InterLabService, Russia). RNA extraction and RT-PCR were performed at the coordinating site to detect the influenza A (H3N2), A(H1N1)pdm09, and B (Yamagata and Victoria lineages) viruses. The anonymized data from the core

questionnaires were collected at the coordinating site and checked for missing, inconsistent, or incorrect data. Only the samples taken within 7 days of the onset of symptoms were included in the study.

RT-PCR

RNA was isolated from 150–200 μl of sample resuspended in a virus transport medium using an AmpliSense® RIBO-prep kit or QIAGEN RNeasy Mini kit. PCR for influenza A and B viruses was performed using AmpliSense® Influenza virus A/B-FL kit. Influenza A-positive samples were used for the subtyping of influenza viruses A(H1N1)pdm09 and A(H3N2) using AmpliSense® Influenza virus A/H1–swine-FL and AmpliSense® Influenza virus A type FL kits. Reverse transcription of RNA was performed using Reverta-L kit (InterLabService, Russia) or QIAGEN OneStep RT-PCR Kit with primers and probes obtained from the Centers for Disease Control and Prevention (CDC Atlanta, GA, USA). Determination of the lineage (Yamagata or Victoria) was performed for all influenza B virus positive specimens using QIAGEN OneStep RT-PCR Kit with WHO recommended primers and probes. Detection of human respiratory syncytial virus (RSV), metapneumovirus, parainfluenza virus, coronavirus, rhinovirus, bocavirus was performed by analysis of cDNA obtained from specimens using AmpliSense® ARVI-screen-FL kit (InterLabService, Russia). Adenovirus B, C, and E groups were also detected by using this system. All RT-PCR procedures were carried out on Rotor-Gene 6000, Corbett Research, Australia or CFX96 Touch™ Real-Time PCR Detection System, BIO-RAD, USA.

Virus isolation

Virus isolation was performed in MDCK cell culture and 10-day-old chicken embryos (CE) incubated for 72 hours at 34°C according to the approved method [11]. Hemagglutination inhibition (HI) test was performed according to the standard method recommended by the WHO, with a 0.75% suspension of human red blood cells (group 0, Rh+) for the influenza A(H3N2) and B viruses. In the case of influenza A(H1N1)pdm09 isolates, a 0.5% suspension of chicken erythrocytes was used [12].

Sequencing

Amplification of cDNA was performed by the standard method using original primers. Sequencing of influenza virus A genome fragments (genes HA, NA, M, and NS) was carried out on ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, USA) with BigDye Terminator Cycle Sequencing Kit v3.1.

Phylogenetic analysis

Processing and analysis of sequences was performed using the software Vector NTI v10.1.1 (Invitrogen) and MEGA 5 (PSU, USA). The method of maximum likelihood (ML) was used to build the phylogenetic trees. The choice of evolutionary models was performed according to the Akaike criterion (AIC) value using the ModelTest v3.7 program.

Statistical analysis

Statistical analysis and data management was performed using Statistica 10.0, including nonparametric statistic. Method Chi-square was used for the determination of significance in the differences of covariates.

RESULTS

Patients recruitment

A total of 1,891 patients with acute respiratory symptoms hospitalized in the 5 wards of three hospitals of St. Petersburg during the periods of increased influenza activity (IAP) for the period from week 3 to week 22 in 2013 and 1,713 patients hospitalized during IAP from week 4 to week 22 in 2014 were investigated. Among them 192 and 422 patients, correspondingly, did not meet the study inclusion criteria and were excluded from further observation. As a result, 1,699 and 1,291 hospitalized ILI-patients were included in the investigation during IAP-2013 and IAP-2014, correspondingly, after obtaining informed consent and initial clinical analysis. Children younger than 17 years old formed the largest group of patients, namely 72.5% and 69.6% of the total number of patients in IAP-2013 and IAP-2014, correspondingly. Children younger than 1 year old and 1 to 4 years old dominated in the study. With the increase of the age, the frequency of children hospitalization decreased. The total number of adult patients was 27.5% and 30.4% in IAP-2013 and IAP-2014, respectively. The number of hospitalized elderly patients was very low during both years. No significant differences in the age distribution among the hospitalized patients were observed between IAP-2013 and IAP-2014 (Table 1).

Comparative data on the etiology of diseases among children and adults

Investigation of clinical samples during the period of IAP in the 2012-2013 and 2013-2014 seasons revealed

positive results for influenza and ARI agents in 52.4% and 58.2% of the investigated patients, respectively. Influenza cases were revealed in 34.7% of the patients in IAP-2013 and only in 15.1% in IAP-2014 when influenza activity in the city was low by both epidemiological and laboratory criteria. Influenza A(H1N1)pdm09 and A(H3N2) diagnoses were confirmed significantly more often among adults from 18 to 84 years old than among children (Table 2). Influenza A(H1N1)pdm09 and influenza A(H3N2) viruses were causative agents of hILI in IAP-2013 while influenza A(H3N2) virus dominated in IAP-2014. Influenza B viruses (mainly the Yamagata lineage) caused hILI cases both in children and in adults in IAP-2013 but was very rarely detected in the patients in IAP-2014.

ARI agents, on the other hand, were detected more often in IAP-2014 than in IAP-2013 (40.8% and 12.7% of investigated patients, respectively), affecting mostly children. During both seasons, RSV was the most often causative agent of hILI among children, dominating among the other non-influenza respiratory agents (Fig. 1). In IAP-2013 this virus caused 13.5% of all hILI in children younger than 1 year old and 5.9% among the group of children 1 to 4 years old while in IAP-2014 these numbers reached 40.2% and 22.8%, respectively. At the same time, RSV played a significant role in etiology of illnesses among the elderly patients. The second significant pathogen was rhinovirus, although its activity was several times lower than that of RSV during both IAPs. Rhinovirus, as well as RSV, more often affected children and elderly people. Parainfluenza and coronavirus were detected very rarely in IAP-2013 but somewhat more often in IAP-2014 (Table 2). Metapneumovirus and bocavirus were rarely observed as causative hILI agents in both seasons.

Monitoring of the confirmed influenza cases

First signs of influenza activity in St. Petersburg in 2013 and 2014 were registered on week 3 and 4 of the

Table 1. Distribution of the patients included in the St. Petersburg study by age groups.

| Patients | Age group (years) | Number of patients investigated | |
|----------------|-------------------|---------------------------------|--------------|
| | | 2013 | 2014 |
| Children | Less than 1 year | 533 (31.4 %) | 368 (28.5%) |
| | 1-4 | 562 (33.1%) | 416 (32.2%) |
| | 5-17 | 144 (8.5%) | 115 (8.9%) |
| | Subtotal children | 1,239 (72.5%) | 899 (69.6%) |
| Adults | 18-49 | 310 (18.2%) | 305 (23.6%) |
| | 50-64 | 113 (6.7%) | 53 (4.1%) |
| | 65-74 | 18 (1.1%) | 11 (0.9%) |
| | 75-84 | 17(1.0%) | 22 (1.7%) |
| | 85 and older | 2 (0.1%) | 1 (0.1%) |
| | Subtotal adults | 467 (27.5%) | 392 (30.4%) |
| Total patients | | 1,699 (100%) | 1,291 (100%) |

Table 2. Comparison of hILI etiology in different age groups of hospitalized patients during two consecutive periods of influenza activity in St. Petersburg.

| Study period | Age group (year) | Patients number | Percent of cases with positive tests for virus ¹ | | | | | | | | | |
|----------------------------|------------------|-----------------|-------------------------------------------------------------|------|--------|--------|-----|-----|------|-----|------|-------|
| | | | H1N1 pdm 09 | H3N2 | B /Yam | B/ Vic | PIV | AdV | RSV | CoV | RV | Total |
| 2013 week 3- week 22 | Up to 1 | 533 | 8,1 | 4,5 | 6,9 | 0,6 | 1,3 | 1,7 | 13,5 | 0,8 | 1,3 | 42,8 |
| | 1 - 4 | 562 | 10,1 | 8,7 | 8,4 | 0,9 | 1,4 | 2,5 | 5,9 | 0,4 | 4,4 | 48,2 |
| | 5 - 17 | 144 | 9,0 | 5,6 | 25,7 | 1,4 | 1,4 | 2,1 | 2,1 | 1,4 | 4,2 | 58,3 |
| | 18 - 49 | 310 | 24,5 | 10,6 | 17,1 | 4,5 | 0,3 | 0,0 | 1,3 | 0,0 | 2,3 | 65,8 |
| | 50 - 64 | 113 | 23,9 | 13,3 | 23,0 | 0,0 | 0,0 | 1,8 | 0,0 | 0,0 | 0,0 | 70,8 |
| | 65 - 74 | 18 | 16,7 | 27,8 | 11,1 | 5,6 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 61,1 |
| | 75 - 84 | 17 | 23,5 | 11,8 | 17,6 | 0,0 | 0,0 | 0,0 | 5,9 | 0,0 | 0,0 | 64,7 |
| | > 85 | 2 | 0,0 | 0,0 | 50,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 50,0 |
| | Total | 1699 | 13,1 | 8,0 | 12,1 | 1,5 | 1,1 | 1,6 | 6,7 | 0,5 | 2,6 | 52,4 |
| 2014 week 4- week 22 | Up to 1 | 368 | 2,2 | 5,7 | 0,0 | 0,0 | 3,8 | 1,1 | 40,2 | 5,2 | 10,6 | 72,6 |
| | 1 - 4 | 416 | 1,9 | 9,6 | 0,2 | 0,0 | 8,2 | 2,6 | 22,8 | 3,8 | 11,1 | 66,1 |
| | 5 - 17 | 115 | 4,3 | 17,4 | 1,7 | 0,0 | 1,7 | 2,6 | 10,4 | 1,7 | 11,3 | 54,8 |
| | 18 - 49 | 305 | 1,0 | 19,0 | 2,0 | 0,0 | 1,0 | 1,0 | 1,6 | 0,7 | 7,5 | 36,1 |
| | 50 - 64 | 53 | 0,0 | 18,9 | 7,5 | 0,0 | 1,9 | 0,0 | 0,0 | 1,9 | 5,7 | 39,6 |
| | 65 - 74 | 11 | 0,0 | 18,2 | 9,1 | 0,0 | 0,0 | 0,0 | 9,1 | 0,0 | 9,1 | 45,5 |
| | 75 - 84 | 22 | 4,5 | 18,2 | 4,5 | 0,0 | 9,1 | 0,0 | 4,5 | 0,0 | 0,0 | 45,5 |
| | > 85 | 1 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| | Total | 1291 | 1,9 | 12,0 | 1,2 | 0,0 | 4,3 | 1,6 | 20,3 | 3,1 | 9,7 | 58,2 |

¹ Virus abbreviations: B/Yamagata (B/Yam), B/Victoria (B/Vic), parainfluenza (PIV), adenovirus (AdV), respiratory syncytial virus (RSV), coronavirus (CoV), rhinovirus (RV).

Note: The most affected age groups by influenza viruses marked in gray, affected by other respiratory viruses marked in green color.

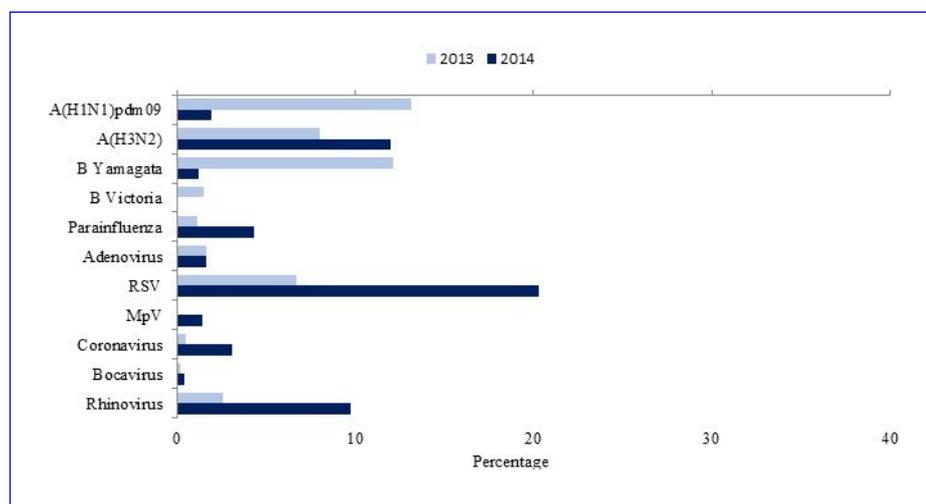


Fig.1. Change of dominating influenza viruses by season and increase of RSV and rhinovirus activity in the season 2013-2014.

corresponding IAPs. During the following weeks of 2013, the rate of the diagnosed influenza A(H1N1)pdm09 cases increased rapidly. The number of patients, both children and adults, with isolated influenza A(H3N2) and B viruses, on the contrary began to increase in the second part of epidemic period. Influenza activity was higher in the epidemic of 2013 in comparison with 2014 when influenza A(H1N1)pdm09 virus was isolated rarely and only weak growth of influenza A(H3N2) activity was observed. Decrease of influenza cases in both seasons was observed after week 17 and the investigation was

stopped on the week 22, when the last single influenza cases were detected (Fig.2).

On the other hand, against the background of low influenza activity, RSV infection burden became very high for the period from week 5 to week 17 of 2014, especially among children. The proportion of influenza, RSV and rhinovirus among all hospitalized patients was 39.6%, 6.7%, and 2.6% in IAP-2013, and 17.4%, 20.3% and 9.7% in IAP-2014, respectively.

Interestingly, in the period of high influenza activity (IAP-2013) the contribution of ARI agents was

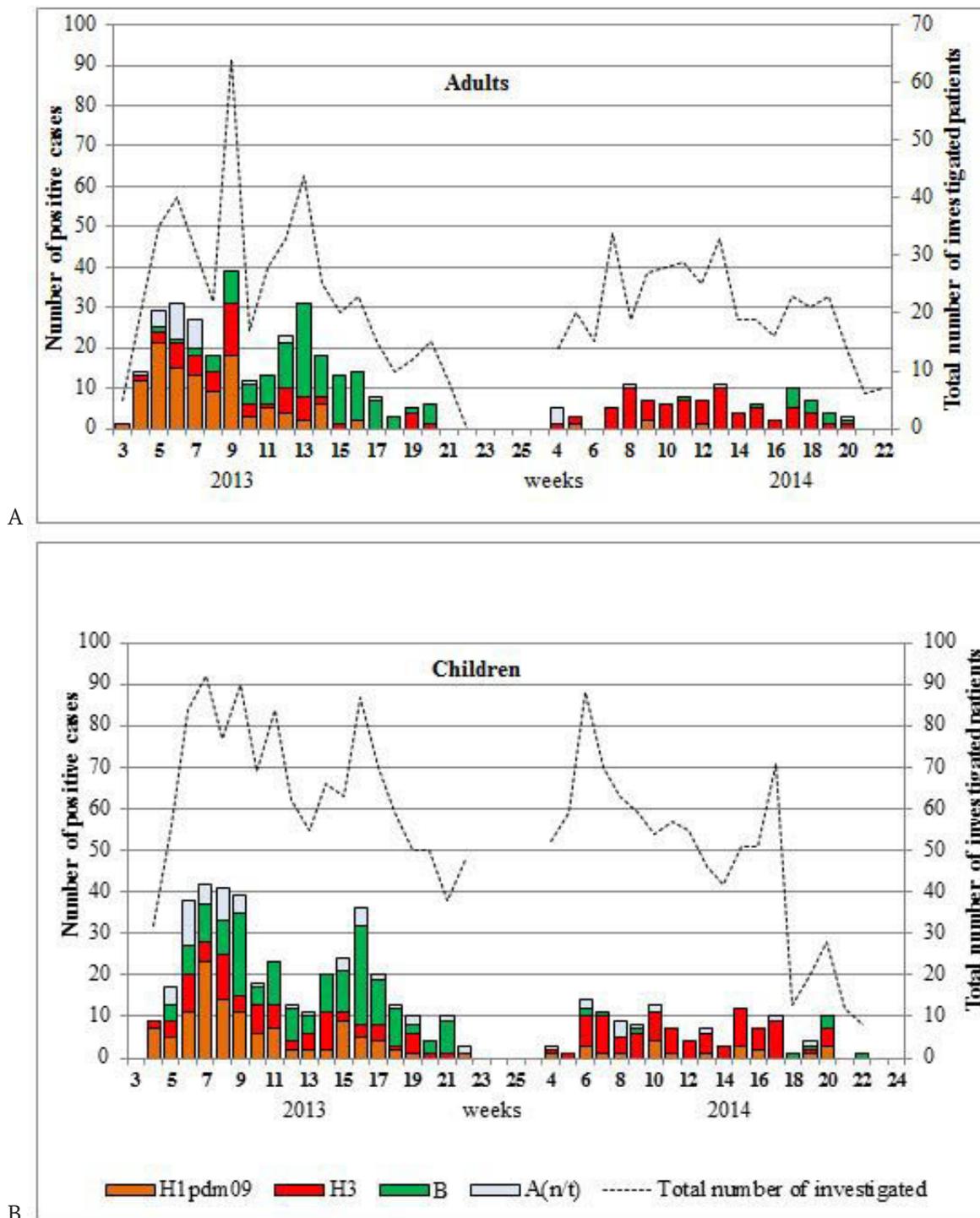


Fig. 2. Etiological monitoring of influenza among children (A) and adult (B) patients hospitalized for the period from week 3 to week 22, in the 2012- 2013 and 2013-2014 seasons.

insignificant and *vice versa*; in the season with low influenza activity (IAP- 2014) the role of ARI agents as a cause for the patients' hospitalization notably increased.

It should be noted that even in the season of low influenza activity, influenza viruses dominated among hospitalized adults; at the same time, RSV and rhinovirus, however, prevailed among children. The total percent of ARI reached 62.8%, 52.2%, and 27.8% among the children of 0 to 2, 3 to 6, and 7 to 14 years old, respectively (Table 2). It is interesting that IAP coincided with RSV increased activity in 2014 although such a clear tendency was not observed in 2013.

During both seasons, the number of male hospitalized patients with ILI was higher than the number of females (15.9% and 12.9% in IAP-2013 and IAP-2014 IAP, respectively), but female patients had more often influenza A and B viruses, especially infant girls younger than 1 year old and 1 to 4 years old, than boys of these age groups.

Etiology of SARI among the patients hospitalized in ICU

In total, 11 patients (0.65% of the total number) and 18 patients (1.4% of the total number) were admitted to ICU in IAP-2013 and IAP-2014, respectively. Most of ICU patients (5 in 2013 and 15 in 2014) were young children younger than 3 months old while the others (with one exception) were adults. Virus etiology of SARI among young children placed in ICU was associated with RSV in 7 cases (35.0% of the total number of cases for both seasons) and with rhinovirus in 3 cases (15.0%), correspondingly. Etiology of SARI among adults placed in ICU was associated with the dominating influenza viruses, namely two cases with influenza A(H1N1)pdm09 and one case with influenza B (Yamagata lineage) in IAP-2013 and only one case with influenza A(H3N2) in IAP-2014. The main reasons for ICU admission of patients were pneumonia, respiratory failure, hyperthermia, or heart failure.

Clinical diagnosis and symptoms of laboratory confirmed influenza and other respiratory viruses among the hospitalized patients

Analysis of the admission data for hospitalized patients was conducted first for IAP-2014. We revealed that the most often diagnosis at admission was "Acute upper respiratory infection" (AURI), namely 27.2%, 21.9%, and 40.9% of total cases for the International codes J06, J06.8 and J06.9, respectively. Several patients were hospitalized with acute tonsillitis (5.8%) and pneumonia (3.1%). Other diagnoses were registered sporadically.

Influenza viruses of A(H1N1)pdm09 subtype in IAP-2013 or A(H3N2) in 2014, RSV, and rhinoviruses dominated as etiological agents independently from primary clinical diagnosis. The exceptions were the acute tonsillitis cases where adenovirus and rhinovirus were detected as the causative pathogen in addition to influenza. It was only in one pneumonia case that the causative agent was proved to be metapneumovirus.

The main diagnoses at admission for the children 0 to 2 years old were AURI and stenosis of the larynx; for the group of children 3 to 6 years old - AURI, bronchitis and pneumonia and for the group 7 to 17 years old - AURI, bronchitis and otitis with sinusitis. A similar pattern of clinical diagnoses (AURI, bronchitis and pneumonia) with the addition of tonsillitis was observed among adult patients 18 to 64 years old, although among the 65 year old patients, pneumonia and bronchitis with cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD), as comorbidities, were the main reasons for hospitalization.

The most common symptoms for influenza among the young children included fever, malaise, cough, and rarely dyspnea; in groups of children 3 to 6 and 7 to 17 years old and in adults these were sore throat, headache, and sometimes myalgia, while dyspnea was observed extremely rare. It should be noted that most of these clinical symptoms were revealed by both the patients with influenza and the patients with negative PCR-confirmed diagnose for influenza and, therefore, cannot be considered as a hallmark of influenza.

Concomitant chronic conditions

Heart disease (HD). During IAP-2013 and IAP-2014, HD was diagnosed in 88 and 64 patients, respectively, and in both periods the percent of HD cases was the highest among the patients 50 years and older (86.6% and 76.6%, respectively). During both periods, the influenza A virus predominantly affected this age group (50 years and older), while influenza B virus was detected more often in groups of younger patients. The average percent of influenza cases among patients with HD was higher in IAP-2013, when influenza A(H1N1)pdm09 dominated, in comparison to IAP-2014, when influenza A(H3N2) prevailed, (60.2% and 25.0%, respectively). On the contrary, the contribution of the other ARI agents increased in IAP-2014 when compared to IAP-2013 from 1.1% to 18.8% with the predominance of rhinovirus (7.8%) and RSV (6.3%) and rear occurrence of parainfluenza (3.1%) and coronavirus (1.6%).

COPD. COPD was diagnosed mostly among the patients 50 years old and older. In total, 58.8% and 35.3% COPD complications among the patients hospitalized with ILI were associated with influenza A in IAP-2013 and IAP-2014, respectively. Influenza B caused this complication only in IAP-2014 (in 5.9% cases). COPD associated with non-influenza respiratory viruses (mostly rhinovirus and parainfluenza) was detected only in 2014 in 17.6% of patients.

Asthma. Asthma was diagnosed among 1.0% and 1.6% of all the hospitalized patients with ILI in 2013 and 2014, respectively. In IAP-2013, asthma was associated with influenza A in 35.3% of the cases (mostly with A(H1N1)pdm09 virus) and in 11.8% cases with influenza B viruses (equally distributed between the Yamagata and Victoria lineages). Rhinovirus was isolated in 11.8% and adenovirus in 5.9% of asthma cases. In IAP-2014, influenza A and B viruses were detected rarely: in 14.3% and 4.8%, respectively. On the other hand, the activity of

other respiratory viruses increased, namely rhinovirus, RSV and coronavirus were dominating (14.3%, 9.5%, and 4.8%, respectively).

Diabetes. Diabetes was diagnosed as a complication in 23 (1.3%) hILI cases in IAP-2013 and in 12 (0.9%) cases in IAP-2014. Influenza A and B viruses were isolated from those patients who had this complication, in 39.1% and 17.4% (only Yamagata lineage), respectively, in IAP-2013 and in 33.3% (mostly H3N2) in IAP-2014. Parainfluenza virus (in 8.3% cases) and no influenza B virus were isolated from patients with diabetes in IAP-2014.

Immunodeficiency (ID). ID as a complication from ILI was diagnosed among 9 (0.7%) patients only in IAP-2014. 22% of ID cases were caused by influenza A(H3N2) infection and 22.2% by rhinovirus infection. No other respiratory agents were isolated among patients with ID complication.

Genetic and antigenic characteristics of influenza A and B viruses isolated in St. Petersburg

It is well known that influenza vaccine efficacy depends on the antigenic matching of the vaccine strains to the circulating viruses. This fact determines the need to monitor the drift of influenza virus in order to adjust the vaccine composition. We obtained and antigenically characterized 230 influenza viruses in total, including 168 influenza A viruses: 135 strains of influenza A(H3N2) and 33 strains of influenza A(H1N1)pdm09, and 62 influenza B viruses: 45 strains of Yamagata and 17 strains of Victoria lineage. All of these viruses were isolated from specimens collected in St. Petersburg during IAP-2013 and IAP-2014. Besides HA and NA, M genes of 45 influenza A and B strains, isolated from these specimens, were sequenced and analyzed.

A(H3N2) viruses. During both epidemic seasons, isolated influenza A(H3N2) viruses revealed decreased titers in HI with ferret and rat antisera to the A/Victoria/361/2011 strain, but did not differ from the reference strain A/Texas/50/2012 included in vaccine composition in Russia.

According to HA genetic analysis all sequenced influenza A(H3N2) viruses belonged to the A/Victoria/361/2011 clade and had amino acid substitutions S45N, T48I, A198S, V223I, and N312S in HA1 and N158D in HA2 subunits specific for the genetic group 3C. Substitutions of 3C.3 subgroup Q33R, N278K, N145S, T128A (leading to the loss of potential site of glycosylation) and R142G were also detected (similar to reference strain A/Samara/73/2013) (Fig. 3). Only one strain, isolated in the end of IAP-2014, belonged to subgroups 3C.2a with substitutions L3I, N144S (loss of potential glycosylation site), F159Y, K160T (appearance of potential glycosylation site), V186G, N225D, Q311H, and D160N.

Thus, most of the analyzed influenza A(H3N2) viruses were drift-variants of A/Victoria/361/2011 virus by their genetic properties and were closely antigenically related to influenza virus A/Texas/50/2012.

A(H1N1)pdm09 viruses. Most of the analyzed influenza A(H1N1)pdm09 viruses were A/California/07/2009-like and reacted well with the antisera to influenza A/South Carolina/20/2010, A/Christchurch/16/2010, A/Hong Kong/5659/2012 and to domestic reference-strains A/St. Petersburg/27/2011 and A/St. Petersburg/26/2013. These data illustrate a very slow antigenic drift of influenza A(H1N1)pdm09 virus despite the gradual accumulation of point mutations in HA gene.

All the A(H1N1)pdm09 viruses, sequenced from original clinical samples obtained in 2013-2014, belonged to group 6 (A/St.Petersburg/27/2011-like) and were attributed to the separate subgroup 6B (A/South Africa/3626/2013) bearing the amino acid substitutions K163Q (Sa), K283E, and A256T in HA1 and E172K in HA2 subunits. The HA sequence of virus in the original clinical sample A/St.Petersburg/R11469S/2014 also contained substitutions V167I, A181S in HA2. (Fig. 4).

Influenza B viruses. The co-circulation of influenza B viruses of two lineages was observed in St. Petersburg in 2013-IAP with the domination of viruses from the Yamagata lineage. Influenza B viruses have not undergone substantial antigenic change. Thus, Victoria viruses retained the antigenic relationship to B/Brisbane/60/2008 reference strain recommended for the composition of influenza vaccine in 2009-2012 seasons. Viruses of the Yamagata lineage of the 2012-2013 season were antigenically similar to the reference strains B/Bangladesh/3333/2007 and B/Wisconsin/1/2010, but some isolates had reduced titers in HI test with antisera to influenza B/Wisconsin/1/2010 strain. In the IAP-2014, only influenza B viruses of the Yamagata lineage were isolated in St. Petersburg. These strains differed significantly in the HI test from the vaccine strain B/Massachusetts/2/2012 with an eightfold reduction of antibody titer to this virus. According to genetic analysis, HA of influenza B viruses of Yamagata lineage isolated in IAP-2013 belong to clade 2 (B/Brisbane/03/2007-like) and have amino acid substitutions R48K (in antigenic site BC), I150S (in BA site), Y165N (in BB2 site), and T181A (in BD site) (Fig. 5). Influenza strains isolated in 2014 as well as viruses isolated from the swabs of hospitalized patients, belonged to clade 3 (B/Wisconsin/01/2010-like) and had 10 amino acid substitutions. Four of these substitutions were in the antigenic sites: N116K (BC), S150I (BA), N165Y (BB2), A181T (BD). These substitutions indicated the reappearance in the circulation of viruses isolated earlier (in 2011-2012 season) that proves the necessity of the replacement of the Yamagata lineage influenza B virus in vaccine composition for the forthcoming season, at least for Russia.

No specific distinctions related to clinical signs and severity of influenza disease were revealed in the HA structure of all the studied viruses. Mutations related to the neuraminidase inhibitors' resistance were not detected in the studied viruses, but all the studied influenza A viruses were resistant to adamantanes according to their M gene structure.

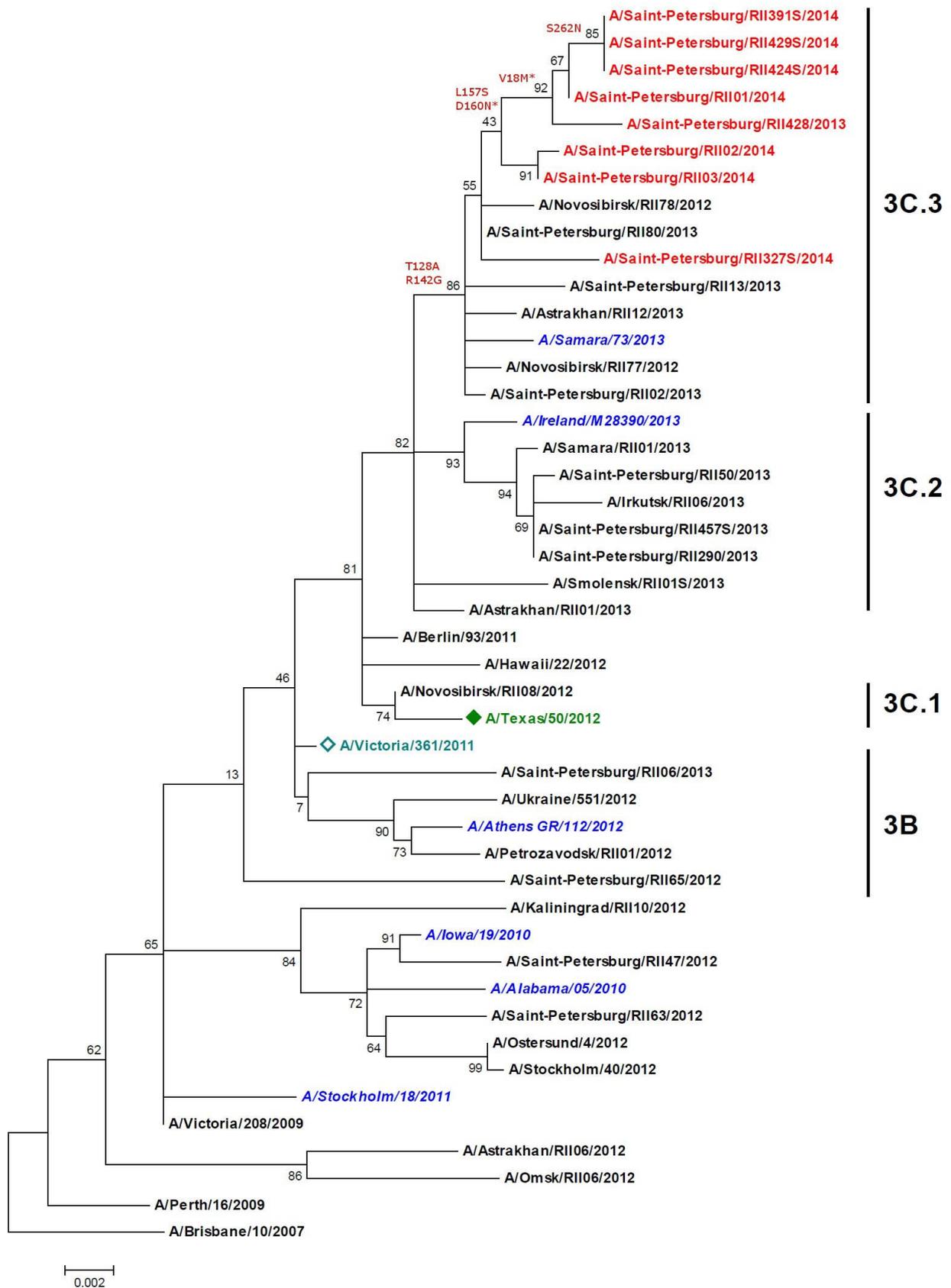


Fig. 3. HA phylogenetic tree of influenza A(H3N2) viruses isolated in the 2012-2013 and 2013-2014 seasons from hospitalized patients with ILI symptoms. Vaccine strains are shown in green (actual season) or blue (previous season). Reference viruses are shown in dark blue. Viruses sequenced in the Research Institute of Influenza are shown in red.

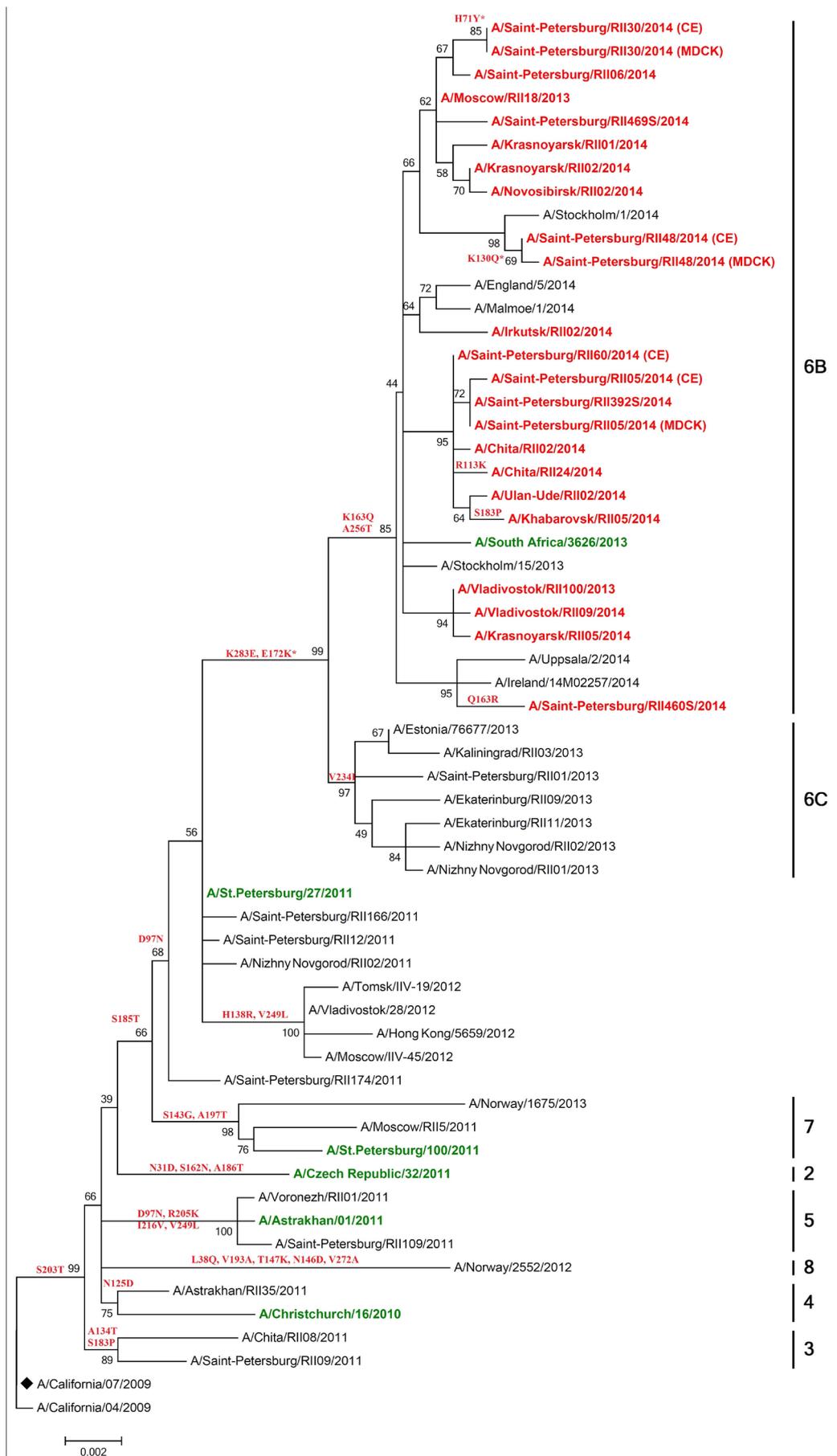


Fig. 4. HA phylogenetic tree of influenza A(H1N1)pdm 09 viruses isolated in the 2012-2013 and 2013-2014 seasons from hospitalized patients with ILI symptoms. Reference viruses are shown in green. Viruses sequenced in the Research Institute of Influenza are shown in red. ♦ – vaccine viruses.

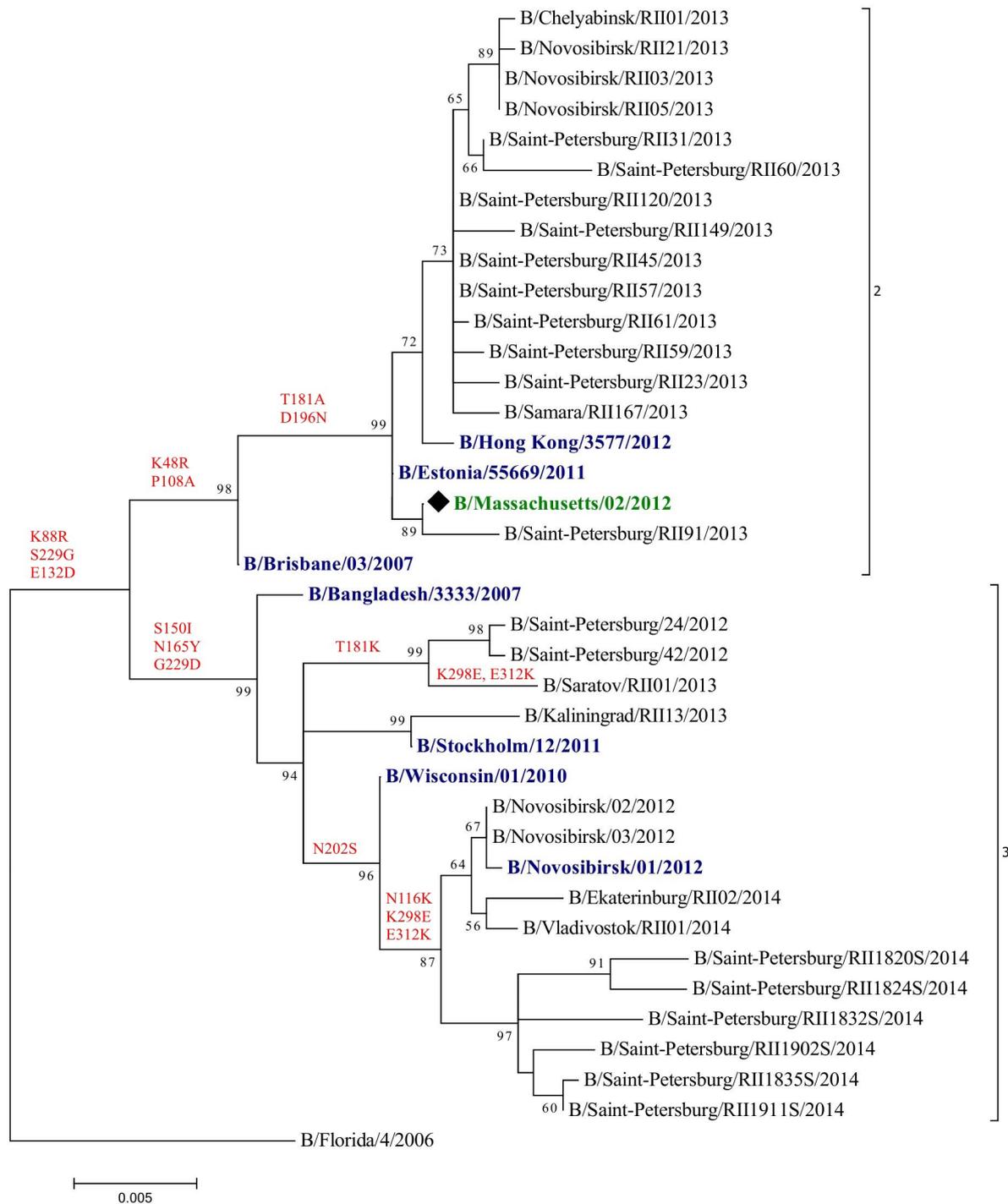


Fig. 5. HA phylogenetic tree of influenza B viruses of the Yamagata lineage isolated in the 2012-2013 and 2013-2014 seasons from hospitalized patients with ILI symptoms. Reference viruses are shown in dark blue. ♦ – vaccine virus.

DISCUSSION

This investigation contributed to the creation of the platform for international state-private partnership in order to find solutions for the clinical and epidemiological problems of influenza infection. The rate of influenza infection in comparison with other ARI viruses among hospitalized children and adults was investigated. Using a RT-PCR diagnosis, we demonstrated that influenza viruses were the dominant causative agents of hILI among the adult patients during the 2013 and 2014 influenza seasons. In IAP-2013, influenza A(H1N1)pdm09 and B (Yamagata lineage) viruses prevailed as the etiological agents of hILI cases. Illnesses, caused by influenza viruses of A(H3N2) subtype and B (Victoria lineage), that led to patient hospitalization, occurred much more rarely in both periods. Conversely, in IAP-2014 cases involving A(H3N2) influenza viruses were observed predominantly while the activity of influenza B viruses was low and only viruses belonging to Yamagata lineage were isolated in this period. These data are consistent with the results obtained in other cities of Russia and in the most of other European countries. The rate of influenza virus infection among children was lower in comparison to adults. In both seasons, the important role of RSV and rhinovirus was determined among the groups of hospitalized with ILI young patients, especially in the groups of children younger than 1 year old and 1 to 4 years old. The number of cases with isolated parainfluenza, adenovirus, metapneumovirus and coronavirus was lower and was minimal for bocavirus.

We showed the important role of influenza viruses in the development of complications among the hILI patients - adults (pneumonia and bronchitis) as well as among children (stenosis of larynx, pneumonia). According to the clinical data, chronic comorbidities such as CVD, COPD, diabetes, and immunodeficiency were developed mainly among adult patients 50 years old and older.

Most of the ICU patients (69.0%) were children younger than 3 months old during both epidemic seasons. The etiology of ILI for children, admitted to ICU, was associated with RSV and rhinovirus. Among the adult ICU patients, only influenza viruses were detected.

The most common diagnosis at the time of patients' admission to the hospital was acute upper respiratory infection; in some cases, acute tonsillitis and pneumonia were diagnosed. Influenza, RSV, and rhinoviruses dominated as the etiological agents of hILI independently from clinical diagnosis. An exception occurred in one case

with a diagnosis of acute tonsillitis in which adenovirus and rhinovirus were detected in addition to influenza.

According to the results of antigenic and genetic analysis, most of the circulated influenza A viruses matched the vaccine strains recommended by the WHO for the 2012-2013 and 2013-2014 seasons. A very slow antigenic drift of influenza A(H1N1)pdm09 virus was noticed despite the gradual accumulation of point mutations in HA gene. Influenza A(H3N2) strains antigenically were similar to A/Texas/50/2012 influenza virus and according to phylogenetic analysis belonged to the clade of A/Victoria/361/2011 (subgroup 3C.3). Most of analyzed strains had two-three amino acid substitutions in HA antigenic sites. At the same time, most of isolated in IAP-2014 influenza B viruses of Yamagata lineage differed antigenically from the vaccine strain B/Massachusetts/2/2012 and were divided to 2 groups. The first group was similar to clade 2 (B/Brisbane/03/2007-like) viruses, while the second group - to the viruses of clade 3 (B/Wisconsin/01/2010-like).

The obtained results emphasize the necessity of expanding the existing influenza surveillance system and ARI in Russia with the inclusion of profound hospital surveillance based on laboratory confirmed etiology. This will help to strengthen the influenza surveillance system in order to provide a forewarning of influenza outbreaks and the emergence of novel influenza viruses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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