Molecular Epidemiology Of Human Influenza Viruses In Thessaly During 2014-2015

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Citation

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DOI: 10.5580/IJLM.34372

Abstract

Influenza virus genotypes circulating in the Thessaly region of central Greece during the 2014-15 season were identified as genetically and antigenically similar to vaccine like viruses. Influenza A (H3N2) viruses were detected and isolated during December January and early February in 63% of the total number of positive for Influenza A and B samples. Influenza B viruses were detected and isolated during February and March 2015. There was found 100% correlation between rt-RT-PCR and cell culture results in the samples assayed. Sequence analysis of the hemagglutinin gene segment revealed a 97% homology to reference viruses in Genbank. These findings support the need for continuous influenza surveillance and underline the importance of influenza vaccine formulation according to the antigenic profile of circulating viral strains.

INTRODUCTION

Influenza viruses constitute important pathogens of the respiratory causing annual epidemics of acute illness and increased indices of morbidity and mortality, worldwide (2, 3). Influenza viruses contain a segmented RNA genome of negative polarity, making these viruses prone to frequent mutation and genetic reassortment events. The latter can occur during infection of a host by viral strains originating from different animal species (1, 4).Immune pressure by the host organism and random mutations acting on hemagglutinin (HA) and neuraminidase (NA) virus surface antigens leads to antigenic drift and the need for the periodic reformulation of the influenza vaccine (5, 6).

Routine screening of respiratory samples for influenza virus detection by a molecular assay combined with sequencing of specific virus gene regions, provides both rapid diagnosis and molecular analysis of viral isolates. Continuous laboratory diagnosis of influenza viruses combined with virus isolation and molecular analysis of viral strains promotes improved management of influenza epidemics via the reformulation of influenza vaccine and development of more efficient antivirals (8).

In the present report we present the first molecular epidemiological study of influenza viruses circulating in central Greece as it relates to influenza activity in the region reported by the sentinel network to the local and state health authorities.

MATERIALS AND METHODS

Clinical samples

During the influenza season in the Thessaly region of central Greece, from December 2014 until March 2015, 750 pharyngeal swabs were examined for the presence of influenza virus. These samples originated from non-vaccinated individuals of all ages presenting with influenza like illness (ILI), 3-4 days following the onset of symptoms. Samples were collected and diagnosed at the University Hospital, Larissa, Thessaly, Greece. Symptoms included fever (>38.50C), myalgia, cough, headache, and malaise. All samples were collected into 2.5 ml virus transport medium (24,9 gr bacto-tryptose, 5 gr gelatin, 0,5 gr streptomycin, 500,000 U penicilin and 50 mg fungizone per liter). Samples were examined immediately or stored at +40C for up to 4 days.

Real-time-RT-PCR (rt-RT-PCR)

Genotyping was performed via an rt-RT-PCR protocol as follows: Total RNA was extracted from 200ml of processed clinical sample with TRIzol (Gibco BRL, Life Technologies, NY, USA), according to manufacturer's recommendations, in the presence of 20mg glycogen, molecular biology grade (Boehringer, Germany). Reactions in real-time RT-PCR were performed in a 7500 Applied Biosystem thermocycler, at a total reaction volume of 25μl (including 6.5μl RNA). Five different master mixes were prepared for each sample (influenza type A, influenza A (H1N1), A(H3N2), influenza type B, and internal control). Forty reaction cycles were performed as follows: 450C for 10 sec, 950C for 10 min and 720C for 1min. Primers and probes used in the reactions are shown in Table 1.

Nucleotide Sequencing

Purified rt-RT-PCR products were sequenced by an automated sequence analyzer CEQ™ 8000 Genetic Analysis System, Beckman Coulter with Genome Lab™ Dye Terminator Cycle Sequencing (Quick Start Kit). Primers used to sequence the HA1 region of the HA gene were as previously reported [7]. DNA sequences obtained were analyzed by the BLAST program (National Center for Biotechnology Information), multiple alignment was performed by the Clustal omega program (European Molecular Biology Laboratory) and virus sequences were compared to influenza virus sequences in Genbank.

Virus isolation and identification

Following rt-RT-PCR screening, all positive to influenza virus samples were propagated in sensitive Mardin-Darby Canine Kidney (MDCK) cell culture and incubated at 37oC for 7 days, as previously described [7]. The Hemagglutination test (HA) was performed on culture supernatants with 0.5% red blood cells and a titer of 1/32 or higher was used for strain identification with the Hemagglutination-inhibition method (HAI) as previously described [7]. Antisera for virus identification were provided by the World Health Organization (WHO) and used according to standard protocol [8].

RESULTS AND DISCUSSION

During the influenza season 2014-15, a total of 750 specimen were assayed for the presence of influenza s A and B gene sequences. There were detected and identified 112 (15%) positive samples by rt-RT-PCR. Type A viruses were detected in 83% of the positive samples assayed, of which 76% were subtype A (H3N2), while the other positive viruses were typed as A(H1N1). Type B viruses were detected during the months February and March 2015, comprising the majority of influenza viruses detected during this two-month period. in central Greece.

The HA1 region of the HA gene segment of selected A (H3N2) and A(H1N1) viral isolates, previously isolated in cell culture and identified by HAI, were analyzed by DNA sequencing and revealed a 97% homology in comparison to reference viruses. Antigenic analysis of the HA antigen of our A (H3N2) and A(H1N1) isolates against vaccine and reference influenza viruses, exhibited a titer of at least 640 indicating antigenic relatedness of our isolates to the vaccine –like viruses. By cell culture method there were isolated both (H3N2, A(H1N1) and B viral strains, with a 100% correlation in comparison to rt-RT-PCR (data not shown).

Influenza activity in the Thessaly region of central Greece was dominated by influenza A viruses, genetically and antigenically similar to the vaccine viruses of the respective season (9). The timely detection and identification of circulating influenza viruses with epidemic or pandemic potential plays a major role in improved influenza vaccine formulation and the development of more efficient anti-viral agents.

Table 1

Primers and probes used in real-time RT-PCR

Influenza virus type/ subtype	Gene target	Primer	Probe
A	matrix gene	Fw CTTCTAACCGAGGTCGAA ACGTA Rv GGTGACAGGATTGGT CTTGTCTTTA	5'-Fam - TCAGGCCCCCTCAAAGCCGAG-3'
B	matrix gene	Fw ATTGCTGGTTTCTTAGA AGG Rv TTGTTTATRGCTTCTTG GGT	5'-Fam- ATGGGAAGGAATGATTGCAGGT-3'
H1N1	HA gene	Fw GAGCTAAGAGAGC AATTGA Rv GTAGATGGATGGTGAA TG	5'-Fam- TTGCTGAGCTTTGGGTATGA -3'
H3N2	HA gene	Fw AGCAAAGCCTACA GCAA Rv GACCTAAGGGAGGCAT AA	5'-Fam- CCGGCACATCATAAGGGTAACA 3'

ACKNOWLEDGEMENT

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

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