Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 69 (2011) 419–427
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Influenza C virus surveillance during the first influenza A (H1N1) 2009 pandemic wave in Catalonia, Spain☆,☆☆

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Received 19 July 2010; accepted 12 November 2010

Abstract

Although particular attention is paid to influenza A and B virus isolates during influenza surveillance, influenza C virus (FLUCV) coexisted during the first influenza A (H1N1) 2009 pandemic wave during the 2009–2010 season. From 27 April 2009 to 9 May 2010, 12 strains of FLUCV were detected in specimens collected from 1713 nonhospitalized patients with upper respiratory tract illness using a molecular method. Half of the patients with FLUCV infection were older than 14 years. The most frequent symptoms were cough and fever, similar to other viral respiratory infections. Phylogenetic analysis of the hemagglutinin-esterase gene revealed that the strains belonged to the C/Kanagawa/1/76-related and C/Sao Paulo/378/82-related lineages, demonstrating their co-circulation in Catalonia. In addition to regular virological surveillance that provides information about the incidence and the exact role of FLUCV in acute viral respiratory infections in the general population, the genetic lineage identification offers additional data for epidemiological purposes.

Keywords: Influenza C virus; Pandemic wave; Surveillance

1. Introduction

Particular attention is paid to influenza A and B virus isolates during influenza surveillance, especially after the declaration of influenza A (H1N1) 2009 pandemic situation by the World Health Organization in June 2009 (current information at http://www.who.int/csr/disease/swineflu/en/index.html). However, infection with influenza C virus (FLUCV) usually coincides with influenza A and influenza B virus activity.

Little is known about the epidemiology and the clinical impact of FLUCV in the general population in Europe (Gouarin et al., 2008; Manuguerra et al., 1992) and especially in Spain (Calvo et al., 2006; Manuguerra et al., 1994). There are few studies concerning FLUCV infection in Europe since few laboratories provide specific diagnoses of influenza C infection and because of its apparently benign nature. Seroepidemiological studies revealed a wide distribution of FLUCV throughout the world, and the acquisition of antibodies to FLUCV during childhood occurs in most of the cases (Dykes et al., 1980; Gouarin et al., 2008; Homma et al., 1982; Manuguerra et al., 1992; Matsuzaki et al., 2006; Nishimura et al., 1987; O'Callaghan et al., 1980).

The hemagglutinin-esterase (HE) gene of FLUCV has been classically divided into 6 lineages, represented by C/Taylor/1233/47, C/Aichi/1/81, C/Sao Paulo/378/82, C/

☆ All authors declare no conflicts of interest.

☆☆ This work was partially supported by Fondo de Investigación Sanitaria (FIS PI08/0118) from the Spanish Ministry of Health, Agència d'Avaluació de Tecnologia i Recerca Mèdiques (AATRM) (grant 402/02/2008), Red Española de Investigación en Patología Infecciosa (REIPI 06/0008).

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doi:10.1016/j.diagmicrobio.2010.11.006

Kanagawa/1/76, C/Yamagata/26/81, and C/Mississippi/80 (Gouarin et al., 2008; Matsuzaki et al., 2003). Phylogenetic studies of FLUCV strains suggested that strains belonging to different lineages are co-circulating (Matsuzaki et al., 1994, 2003).

People infected with FLUCV may exhibit symptoms with a similar severity to that caused by influenza A and B virus infections. FLUCV usually causes upper respiratory tract infections in children and young adults (Gouarin et al., 2008; Greenbaum et al., 1998; Katagiri et al., 1983; Manuguerra et al., 1992), although it can also cause lower respiratory infections, such as bronchitis, bronchiolitis, or pneumonia (Calvo et al., 2006; Moriuchi et al., 1991).

In addition to regular virological surveillance that provides information about the incidence and the exact role of FLUCV in acute viral respiratory infections in the general population, the genetic lineage identification offers more data for epidemiological purposes.

We present a prospective study of a nonhospitalized general population in whom the incidence and the genetic lineage identification of FLUCV strains detected during the first influenza A (H1N1) 2009 pandemic wave are reported.

2. Materials and methods

2.1. Surveillance system

Influenza Surveillance in Catalonia (PIDIRAC: Pla d'informació de les infeccions respiratòries agudes a Catalunya. Departament de Salut, Generalitat de Catalunya. Available at http://www.gencat.cat/salut/depsalut/html/ca/dir3157/plapidirac.pdf), located in Northeastern Spain, is based on a medical sentinel network that includes 56 physicians from 25 primary care centers which covers approximately 1% of the total population. This network provides a wide geographical coverage of influenza virus incidence in Catalonia, facilitating timely information exchange on influenza activity. Clinical data of the patients are recorded prospectively by sentinel physicians.

2.2. Clinical specimens and virus detection

From 27 April 2009 (week 17/2009) to 9 May 2010 (week 18/2010), nasopharyngeal samples (nasal and oropharyngeal swabs) were collected from patients with upper respiratory tract illness (URTI) and placed in a tube with viral transport medium. Within the first 24 h, they were processed in Biosafety Level 2 Plus (BSL 2+) facilities, being kept at 2–4 °C in several aliquots until use. Total nucleic acids were extracted from 200 μL of fresh specimen and eluted in 25 μL of RNase-free elution buffer using NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Nucleic acids were kept frozen until use.

Two independent multiplex nested reverse transcription polymerase chain reactions previously described (Coiras et al., 2003, 2004) were used to detect human influenza viruses (types A, B, and C) and other human respiratory viruses. In those laboratory-confirmed positive samples for influenza A virus, a specific 1-step multiplex real-time RT-PCR was used for subtyping (H1/H3/H5/pandemic H1) as described (Anton et al., 2010).

2.3. Nucleotide sequencing

The coding region (CDS) from nucleotides 23 to 1989 of the HE gene was sequenced by a PCR-based sequencing method from laboratory-confirmed FLUCV-infected respiratory specimens. Direct PCR sequencing was done using overlapping primer pairs, available upon request, by nested PCR. Purified PCR products using Exo-SAP-IT (USB, Affymetrix, Inc., Cleveland, OH, USA) were sequenced by the ABI Prism BigDye Terminator cycle sequencing kit v. 3.1 on an ABI 3130XL automatic sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were assembled and edited using the SeqScape v. 2.5 software (Applied Biosystems).

2.4. Phylogenetic analysis

Sequences of the present study and representative sequences of each lineage, previously published (Buonagurio et al., 1985; Matsuzaki et al., 1994; 2000; 2002; 2003; 2004; 2007; Muraki et al., 1996; 2004), were downloaded from the GISAID Database and aligned using ClustalX v. 2.0.12 (Larkin et al., 2007) with default parameters. After alignment of the HE sequences without the initial signal peptide sequence (MFFSLLLMLGLTEA, Signal Peptide Database, http://www.signalpeptide.de), ambiguous regions (i.e., containing gaps and/or poorly aligned) were removed with Gblocks (v. 0.91b) (Castresana, 2000) using the following parameters: at least 10-base pair length of a block after gap cleaning, no gap positions allowed in the final alignments, and rejection of segments with contiguous nonconserved positions bigger than 8 bases. The conversion among different file formats of multiple sequence alignment was done with ALigment Transformation EnviRonment (ALTER) (Glez-Pena et al., 2010). The molecular evolutionary model of nucleotide substitution was fitted to the multiple sequence alignment using jModelTest v. 0.1.1 (Posada, 2008). The phylogenetic tree was reconstructed using 2 approaches: i) a maximum likelihood method as implemented in the PhyML v. 3.0 program (Guindon and Gascuel, 2003) and ii) a neighbor-joining distance method as implemented in Mega v. 4.1 (Tamura et al., 2007) with the best evolutionary model found previously in jModelTest and which is implemented in each of the programs. Reliability for the internal branch was assessed using the nonparametric bootstrap analysis (500 replicates). Graphical representation and editing of the phylogenetic tree were performed with Tree Explorer (available in Mega v. 4.1).

In order to detect those amino acid substitutions that define clades and subclades, the amino acid sequences