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Short communication

Oseltamivir-resistant pandemic influenza a (H1N1) 2009 viruses in Spain

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ABSTRACT

Background: Pandemic influenza A (H1N1) 2009 virus appeared in Spain on April 25, 2009 for the first time. This new virus was adamantane-resistant but it was sensitive to neuraminidase (NA) inhibitors oseltamivir and zanamivir.
Objectives: To detect oseltamivir-resistant pandemic influenza A (H1N1) 2009 viruses by the Spanish Influenza Surveillance System (SISS) and a possible spread of oseltamivir-resistant viruses in Spain since starting of the pandemic situation.
Study design: A total of 1229 respiratory samples taken from 413 severe and 766 non-severe patients with confirmed viral detection of pandemic influenza A (H1N1) 2009 viruses from different Spanish regions were analyzed for the specific detection of the H275Y mutation in NA between April 2009 and May 2010.
Results: H275Y NA substitution was found in 8 patients infected with pandemic influenza A (H1N1) 2009 viruses collected in November and December 2009 and in January 2010. All oseltamivir-resistant viruses were detected in severe patients (8/413, 1.93%) who previously received treatment with oseltamivir. Six of these patients were immunocompromised.
Conclusion: In Spain, the number of oseltamivir-resistant pandemic influenza A (H1N1) 2009 viruses is until now very low. No evidence for any spread of oseltamivir-resistant H1N1 viruses is achieved in our Country.

1. Background

Pandemic influenza A (H1N1) 2009 virus was identified in human population in Mexico and United States in March and early April 2009 for the first time.[1,2] Initial testing of pandemic H1N1 virus found it susceptible to neuraminidase inhibitors, oseltamivir and zanamivir, but resistant to amantadine.[3]

The genetic basis of resistance to adamantanes is associated with certain amino acid substitutions in the transmembrane domain of the Matrix protein (M2) at positions 26, 27, 30, 31 or 34.[4] In addition, oseltamivir and zanamivir bind the active site of the neuraminidase enzyme (NA) in the virion surface. Substitutions described at positions 119, 275 or 295 in the NA of influenza viruses have been proved with a reduction of susceptibility to oseltamivir[5] and also substitutions at positions 119 and 152 have been related with resistance to zanamivir.[5,6] Viruses carried a mutation H275Y (H274Y in N2 numbering) presented structural alterations that weaken oseltamivir binding.[7]

Pandemic influenza A (H1N1) 2009 virus has S31N substitution[8] and studies about antiviral susceptibility have shown majority of viruses are resistant to adamantanes and sensitive to antiviral treat-

Abbreviations: NA, neuraminidase; SISS, Spanish Influenza Surveillance System; WHO, World Health Organization; H275Y, histidine-275-tyrosine; E119V, glutamic acid-119-valine; N295S, asparagine-295-serine; RT-PCR, reverse transcription polymerase chain reaction; GP, general practitioner.

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ment with oseltamivir and zanamivir.[9] However, cases of pandemic influenza A (H1N1) 2009 viruses with H275Y substitution have been reported[9,10] and until now 313 oseltamivir-resistant viruses have been reported around the world.[11]

Surveillance studies based on monitor the appearance of E119V, H275Y or N295S mutations in pandemic H1N1 virus are essential for the public health point of view in our country. This work is based on virological data provided by some laboratories involved in the Spanish Influenza Surveillance System (SISS).

2. Objectives

The aim of this study is to detect oseltamivir-resistant viruses in Spain and the possible spread of these resistant viruses.

3. Study design

To investigate the appearance of oseltamivir-resistant viruses in Spain between April 2009 and May 2010, a total of 1229 respiratory samples from 1179 patients with confirmed pandemic influenza A (H1N1) 2009 virus infection from 16 Spanish regions were analyzed.

At the National Influenza Center in Madrid (Instituto de Salud Carlos III, Spain) and at some other laboratories involved in the SISS, specific detection of the H275Y substitution in the NA protein was investigated in a first group consisting in 461 respiratory samples taken from 413 patients hospitalized with severe clinical disease and in a second group of 768 respiratory samples from 766 patients with mild clinical disease (non hospitalized and sentinel patients). Severe cases were defined as any condition or clinical presentation requering hospital addmision for clinical management according to WHO guidance criteria.[12] Antiviral therapy was used in severe patients according to Spanish Ministry of Health recommendations.[13] Samples were assayed for molecular amplification of partial NA gene by generic primers as previously published[14] with minor modifications. Primers FluARNA+ (5′-TATTCGTCTCAGGGAGCRAAAGCRGG-3′) and FluARNA- (5′-ATATCGTCTCGTATTAGTAGAAACAAGG-3′) were used for RT-PCR and NA1+ (5′-GCAAGYGCWTGYCATGATGGC-3′) and NA1- (5′-CCTCTGATYAIYTCIACIYARAARCA-3′) were used for nested PCR. The presence of H275Y substitution was investigated by analysis of the corresponding sequences or, in some cases, by a simple probe hybridization approach method (LightMix Kit Influenza A Virus HxN1 Tamiflu resistance, TIB MOLBIOL, Berlin, Germany, for Light Cycler 2.0 instrument, Roche Diagnostic,).

4. Results

Sequences analyzed from the majority of pandemic influenza A (H1N1) 2009 viruses studied corresponded to the wild type at position 275 in the NA gene, providing no evidence for any circulation of oseltamivir-resistant pandemic H1N1 viruses in Spain. The H275Y substitution was found in the NA sequences of 8 pandemic influenza A (H1N1) 2009 viruses detected in Andalucia (2), Islas Baleares (1), Galicia (3), Navarra (1) and Pais Vasco (1) in positive respiratory samples collected in November and December 2009 and in January 2010 (Table 1).

All oseltamivir-resistant viruses were detected in severe patients (8/413, 1.93%): six patients needed intensive care and 5 of them were fatal cases. Six patients were immunosupressed and the rest two patients showed chronic kidney failure and mitochondrial disease. All positive resistant virus patients received treatment with oseltamivir during the hospitalization stay. In two patients from Pais Vasco (patient 4) and from Galicia (patient 7) more than one respiratory sample were analyzed after the beginning

Table 1
Characteristics of the patients infected with influenza A (H1N1) 2009 virus having evidence of oseltamivir-resistance (H275Y substitution), Spain 2009–2010.

Patient	Strain	Geographical region	Age (years)	Sex	Beginning of symptoms (dd/mm/yyyy)	Beginning of Oseltamivir therapy (dd/mm/yyyy)	Oseltamivir regimen recommended (dose)	Date of sample collection (dd/mm/yyyy)	NA mutation	Intensive care required	Status	Clinical considerations
1	A/Andalucia/9109664/2009	Andalucia	1	Female	28/11/2009	30/11/2009	Unknown	16/12/2009	275Y	Yes	Dead	Leukemia
2	A/Andalucia/0008187/2010	Andalucia	30	Female	05/01/2010	05/01/2010	4 days (unknown)	21/01/2010	275Y	Yes	Dead	Lymphoma
3	A/Baleares/RR6121/2009	Islas Baleares	3	Male	23/11/2009	25/11/2009	15 days (150 mg/12 h)	30/11/2009	275Y	Yes	Dead	Leukemia
4	A/PaisVasco/547876/2009	País Vasco	2	Female	09/11/2009	12/11/2009	5 days (60 mg/24 h)	15/11/2009 23/11/2009	275H 275Y	Yes	Dead	Best's disease, mitochondrial cytopathy
5	A/Galicia/09-530630/2009	Galicia	8	Male	07/09/2009	08/09/2009	15 days (45 mg/12 h)	18/09/2009	275Y	No	Alive	Hodgkin's linfoma
6	A/Galicia/10-303871/2009	Galicia	29	Female	29/10/2009	30/10/2009	10 days (75 mg/12 h)	07/11/2009	275Y	Yes	Dead	Lung Transplantation
7	A/Galicia/10-300808/2010	Galicia	75	Female	26/12/2009	03/01/2010	20 days (unknown)	09/01/2010 15/01/2010	275H 275Y	Yes	Alive	Lymphoma
8	A/Navarra/RR5989/2009	Navarra	70	Male	30/10/2009	30/10/2009	4 days (75 mg/24 h)	03/11/2009	275Y	No	Alive	Chronic kidney failure