

OSELTAMIVIR SUSCEPTIBILITY IN SOUTH-WESTERN FRANCE DURING THE 2007-8 AND 2008-9 INFLUENZA EPIDEMICS AND THE ONGOING INFLUENZA PANDEMIC 2009

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The recent emergence of seasonal influenza A(H1N1) strains resistant to oseltamivir makes it necessary to monitor carefully the susceptibility of human influenza viruses to neuraminidase inhibitors. We report the prevalence of the oseltamivir resistance among influenza A viruses circulating in south-western France over the past three years: seasonal influenza A(H1N1), seasonal influenza A(H3N2), and the influenza A(H1N1)v viruses associated with the ongoing 2009 pandemic. The main result of the study is the absence of oseltamivir resistance in the pandemic H1N1 influenza strains studied so far (n=129).

Introduction

Even if yearly vaccination remains the best way to prevent influenza, antiviral drugs have proven their efficacy in preventing and treating acute influenza. The adamantanes (amantadine and rimantadine) were the first available influenza antiviral medications. They are associated with severe adverse effects and high levels of resistance among influenza A viruses [1]. This resistance may occur in the absence of antiviral drug use and also emerge rapidly under treatment. Fortunately, neuraminidase inhibitors (NAIs) have been designed to expand the therapeutic possibilities. Presently two anti-influenza drugs are commercially available: oseltamivir and zanamivir [2], which selectively inhibit the neuraminidase of both influenza A and B viruses. Oseltamivir is preferred over zanamivir because it is administered by the oral route [2]. NAIs have been prescribed worldwide since 1999 [3]. In France, their use was limited before the influenza pandemic 2009.

Until recently, the level of resistance to NAIs among circulating influenza A viruses was low [3,4]. However, surveillance studies revealed the sudden emergence of seasonal A(H1N1) strains resistant to oseltamivir in 2007-2008 in Europe where NAIs are used sparsely [5]. From the last quarter of 2007 until June 2008, the highest rate of resistance was reported in Norway (67%). France had the second highest rate with 47% of seasonal A(H1N1) viruses resistant to oseltamivir [6].

Mutations implicated in NAIs resistance were found to be subtype-specific in the neuraminidase active site: The mutations R292K and E119V (in N2 numbering) predominate in the influenza A(H3N2) subtype. R292K induces a resistance to both NAI, whereas E119V leads to oseltamivir but not to zanamivir resistance. H274Y (in N2 numbering) predominates in the seasonal influenza A(H1N1) subtype and confers a high level of resistance to oseltamivir, but these strains remain sensitive to zanamivir [7].

During the season 2007-8, the predominant influenza subtype circulating in south-western France was A(H1N1), while influenza A(H3N2) viruses were the paramount subtype in the 2008-9 winter season. In April 2009, the new influenza A(H1N1)v virus emerged, which has the potential for rapid spread [8]. In the present study, influenza A viruses were collected during two consecutive seasons, 2007-8 and 2008-9, and during the current ongoing influenza pandemic (May to mid-September 2009) for surveillance of oseltamivir resistance using sequence analysis.

Methods

Respiratory samples of patients with influenza-like illness were obtained from Bordeaux Hospital and through a sentinel surveillance network of 21 general practitioners in south-western France. These clinical samples were nasal swabs, bronchoalveolar lavage fluids and nasopharyngeal secretions and were screened by real time RT-PCR in order to determine the virus strain. Primers and probes for the seasonal influenza strains were designed 'in house', those for influenza A(H1N1)v viruses were developed and provided by the two French National Reference Centres for influenza viruses (North and South). None of the patients from whom respiratory specimens were obtained had been treated with NAI before.

The influenza A virus isolates were screened for mutations known to confer resistance to oseltamivir by sequencing of the neuraminidase gene. A multiple sequence alignment was done of influenza A neuraminidase sequences available in Genbank, in order to choose specific RT-PCR primers that would recognise most of the influenza A(H1N1) and A(H3N2) seasonal strains and the pandemic influenza A(H1N1)v. Three primer pairs were designed, targeting the following regions: nucleotide positions 684 to 1,021 of the N1 gene for seasonal influenza A(H1N1) and 692 to 930 for pandemic influenza A(H1N1)v, and nucleotide positions 153 to 1,078 of the N2 gene for seasonal influenza A(H3N2). The target regions were amplified by RT-PCR and sequenced.

The epidemiological features of the ongoing influenza H1N1 pandemic in south-western France were studied following specific instructions from the French Ministry of Health. The target populations were: patients coming from endemic countries (mainly South America and the United States), patients with severe influenza infection, clustered cases of influenza in the community or at school and work place, or pregnant women, children under the age of five months and healthcare workers who had influenza-like symptoms.

Results

In this surveillance study we could amplify sequences for 21 seasonal influenza A(H1N1) viruses in the 2007-8 influenza season, for 97 seasonal influenza A strains (92 H3N2 and five H1N1) in 2008-9, and for 173 pandemic influenza A(H1N1)v viruses collected during the ongoing pandemic. The neuraminidase genes of all 21 seasonal influenza A(H1N1) viruses detected in south-western France during the 2007-8 influenza season were successfully sequenced, and 47.6% of them (10/21) contained a mutation associated with oseltamivir resistance. During the 2008-9 season, none of the 92 seasonal influenza A(H3N2) virus samples contained the E119V or the R292K mutation in the neuraminidase N2 sequence, but all five co-circulating seasonal influenza A(H1N1) viruses had the H274Y mutation in the neuraminidase N1 gene. Since the beginning of the pandemic in late April 2009, 173 confirmed cases of pandemic influenza A(H1N1)v have been found in south-western France. Only 129 of those isolates have been genotyped so far. According to their neuraminidase sequence, all

129 were found to be sensitive to oseltamivir (Table 1). Currently, influenza A(H1N1) 2009 incidence is increasing worldwide including in south-western France (Table 2). As already described, young adults (19-34 years) seem to be particularly sensitive to A(H1N1) 2009 infection (Figure).

Discussion

As we had no phenotypic data in this study, we could not observe potential new mutations leading to resistance. Therefore, this study is limited to previously described resistance mutations that can be shown by sequencing. We report the results of a surveillance study for NAI susceptibility among influenza A viruses isolated in south-western France during the last two influenza seasons and the current 2009 pandemic. Results obtained in the 2007-8 and 2008-9 influenza seasons are in accordance with the World Health Organization's Global Influenza Surveillance Network data. The recent emergence of oseltamivir-resistant influenza A(H1N1) strains during 2007-8 season in western Europe may appear surprising in view of the small proportion of treated patients [9]. This could have dramatic consequences if resistance were to emerge also among avian influenza A(H5N1) viruses or pandemic influenza A(H1N1)v strains. To date, only 12 oseltamivir-resistant influenza A(H1N1)v viruses have been detected worldwide, namely in Canada, China, Denmark, Hong Kong, Japan, Singapore and the United States [10]. Oseltamivir has been recommended since the beginning of the influenza pandemic 2009 for treatment and prophylaxis. Monitoring the susceptibility of pandemic influenza viruses to oseltamivir is important to identify cases in which zanamivir should be used as an alternative drug.

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TABLE 1

Oseltamivir resistance in influenza A isolates collected since 2007 in south-western France (n=247)

	Number of samples genotyped	Number of oseltamivir-resistant samples
2007-2008	21 A(H1N1) seasonal	10
2008-2009	5 A(H1N1) seasonal	5
	92 A(H3N2) seasonal	0
1 May - 15 September 2009	129 A(H1N1) 2009 pandemic	0

TABLE 2

Prevalence of pandemic influenza A(H1N1)v prevalence in south-western France, 1 May to 15 September 2009 (n=173)

	May	June	July	August	Sept.	Total
Number of samples tested	31	36	93	410	302	872
Number of influenza A(H1N1)v cases	3	9	8	113	40	173
Positive ratio (%)	9,7	25,0	8,6	27,6	13,2	19,8

FIGURE

Age distribution of cases of pandemic influenza A(H1N1)v, south-western France, 1 May - 15 September 2009 (n=173)

