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Short communication

Emergence of oseltamivir-resistant influenza A H1N1 virus during the 2007–2008 winter season in Luxembourg: Clinical characteristics and epidemiology

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ABSTRACT

Human cases of oseltamivir-resistant influenza A H1N1 virus emerging in 2007–2008 in Luxembourg were not associated with treatment, prophylaxis or stockpiling of oseltamivir. Following initial local seeding, oseltamivir-resistant strains spread synchronously to sensitive strains causing a similar epidemiology and clinical symptoms.

During the winter of 2007/2008, a high proportion of oseltamivir-resistant isolates of influenza A subtype H1N1 were observed initially in Europe and spread to other continents during 2008–2009 (Besselaar et al., 2008; Influenza Project Team, 2008; Lackenby et al., 2008a; Nicoll et al., 2008; Meijer et al., 2009; Hauge et al., 2009; Dharan et al., 2009). This has caused concerns on the effectiveness of antiviral drugs and the rationale of their national stockpiling as recommended by the World Health Organization and European Center for Disease Control for pandemic planning and preparedness, particular in view of a novel A H1N1 virus likely to cause the next pandemic (Fraser et al., 2009).

We conducted a retrospective analysis of all laboratory confirmed influenza cases in Luxembourg during the seasonal epidemic in 2007/2008 to assess a possible link between the emergence of oseltamivir resistance and prophylactic exposure, treatment or stockpiling of oseltamivir. We also investigated whether patients with oseltamivir-resistant strains differed in terms of clinical symptoms and epidemiologic characteristics from those with sensitive strains.

This study was conducted within the framework of the national influenza sentinel surveillance in Luxembourg. During the winter season, a sentinel of 12 general practitioners and 4 pediatricians sent nose and throat swabs of patients with influenza-like illness (ILI) as well as a weekly summary of clinical data to the National Health Laboratory. In addition, approximately 20% of samples were obtained from non-sentinel doctors (see Table 1). Total RNA was extracted directly from clinical specimens either using QIAamp MinElute Virus Spin kit (Qiagen Benelux, The Netherlands) or NucliSENS easyMAG (Biomérieux, France). Influenza A and B positive samples were detected using the commercially available Influenza Virus (Flu A/B) primer and probe set (ASRFLU-150N-040) on the SmartCycler platform (Cepheid, Sunnyvale, California). Resistance to oseltamivir (substitution of histidine by tyrosine H274Y in the neuraminidase (NA)) in H1N1 strains was determined by pyrosequencing of the corresponding nucleotides in the NA gene (Lackenby et al., 2008b). The 795 terminal nucleotide sequences of NA genes were obtained by cycle sequencing using gene-specific primers, after amplification of the corresponding gene segment by RT-PCR. Our national data, which are kept up-to-date on a web page (Sentinel Surveillance of Influenza, 2009), were forwarded to the European Influenza Surveillance Scheme on a weekly basis.

During the winter season (1st October 2007–1st May 2008) 1040 patient samples were referred to our Laboratory, of which 270 were positive for influenza A and 198 were positive for influenza B. Of the 270 samples positive for influenza A, 195 (72.2%) samples were characterized as oseltamivir-sensitive H1N1, 59 (21.9%) sam-

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Table 1
Comparison of demographic characteristics and clinical features of patients with oseltamivir-resistant influenza A H1N1, oseltamivir-sensitive influenza A H1N1 and influenza B during the 2007–2008 winter season in Luxembourg. All statistical tests were conducted using Stata 10.

Characteristic	Sample size (% of all influenza patients)	Oseltamivir-resistant influenza A H1N1	Oseltamivir-sensitive influenza A H1N1	Influenza B	*p*-Value
Mean age in years (SD[a])	451 (99.8%)	22.1 (15.5)	22.6 (16.8)	24.8 (18.9)	0.391[b]
Female	444 (98.2%)	61%	50%	44%	0.057[c]
Mid point of epidemic (median)	452 (100%)	2008/01/31	2008/01/31	2008/02/22	<0.001[d]
Median maximum temperature (°C)	364 (80.5%)	39	39	39	0.477[d]
Sudden onset	355 (78.5%)	95%	96%	98%	0.331[c]
Shivers	355 (78.5%)	67%	58%	63%	0.465[c]
Headache	355 (78.5%)	76%	79%	84%	0.340[c]
Muscle pain	355 (78.5%)	74%	64%	74%	0.107[c]
Fatigue	355 (78.5%)	74%	60%	72%	0.043[c]
Dry cough	355 (78.5%)	89%	81%	87%	0.251[c]
Sore throat	355 (78.5%)	70%	58%	66%	0.202[c]
Runny nose	355 (78.5%)	65%	72%	76%	0.320[c]
GP member of sentinel	462 (100%)	80%	72%	81%	0.118[c]

[a] SD: standard deviation.
[b] One-way analysis of variance, *F*-test.
[c] Chi-square test on two-way table.
[d] Non-parametric test of equality of medians.

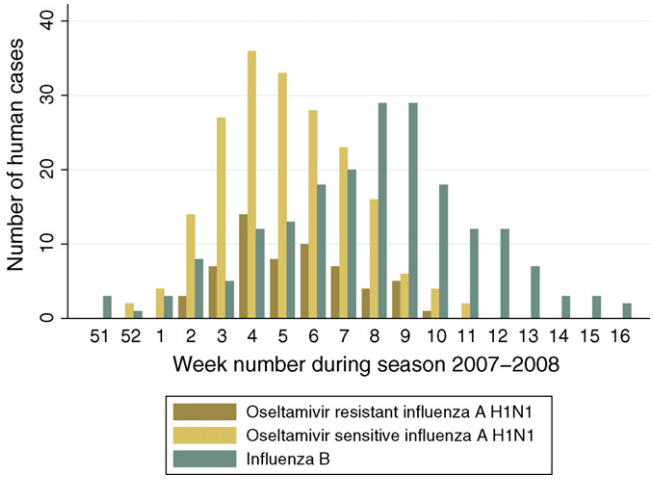

Fig. 1. Epidemic curve of influenza in Luxembourg during the 2007/2008 winter season.

ples as oseltamivir-resistant H1N1 and 7 (2.6%) samples as H3N2 subtypes.

The epidemic curve (Fig. 1) shows that the emergence of oseltamivir-resistant influenza A H1N1 strains was synchronous with the occurrence of oseltamivir-sensitive influenza A H1N1 strains. The proportion of oseltamivir-resistant influenza A H1N1 strains did not change significantly over the course of the season (non-parametric test on difference of median date, $p = 0.95$), but the influenza B epidemic was delayed by three to five weeks in comparison to the influenza A H1N1 epidemic (non-parametric test on difference of median date, $p < 0.001$). Based on the data routinely collected for all samples, Table 1 shows that the characteristics and symptoms of patients infected with oseltamivir-resistant influenza A H1N1 strains were no different from those infected with oseltamivir-sensitive strains of influenza A H1N1 or influenza B strains.

In addition, we conducted a rapid retrospective mail-based survey to collect further information on use and stockpiling of oseltamivir, duration of illness, hospitalization and attack rates within a household. All 282 patients with laboratory-confirmed influenza reported before the month of March were invited to complete a one page paper questionnaire. A total of 212 (75%) patients responded. Results shown in Table 2 indicate no significant difference between patients infected with oseltamivir-resistant and oseltamivir-sensitive influenza A H1N1 or with influenza B strains for any of the variables of interest. While a sizable fraction (11.4%) of all patients reported treatment with oseltamivir during acute illness, only one patient (0.4%) reported having stockpiled oseltamivir prior to the present influenza episode and one (0.4%) patient with

Table 2
Clinical and epidemiologic characteristics of survey respondents. All statistical tests were conducted using Stata 10.

Patient characteristics	Oseltamivir-resistant influenza A H1N1	Oseltamivir-sensitive influenza A H1N1	Influenza B	*p*-Value
Sample size	42	112	57	
Mean age in years (SD[a])	22.2 (15.6)	20.5 (16.3)	8.2 (21.2)	0.858[b]
Female	64%	54%	42%	0.088[c]
Median duration of illness in days	7	6	8	0.058[d]
Median duration of sickness leave from work or school	3.5	4	5	0.255[d]
Hospitalized	0 (0%)	1 (0.9%)	2 (3.5%)	0.282[c]
Vaccinated	0	3 (2.7%)	2 (3.5%)	0.71[c]
Treated with oseltamivir during illness	6 (14.3%)	12 (10.7%)	6 (10.5%)	0.806[c]
Prophylactic use of oseltamivir	0 (0%)	1 (0.9%)	0 (0%)	1.0[c]
Stock of oseltamivir at home before illness	1 (2.4%)	0 (0%)	0 (0%)	0.199[c]
Would accept vaccination in future	15 (35.7%)	26 (23.2%)	18 (31.6%)	0.237[c]
Attack rate in household (number of persons ill during same period/number of persons living in household)	59/149 (39.6%)	141/349 (40.9%)	85/195 (43.6%)	0.702[c]

[a] SD: standard deviation.
[b] One-way analysis of variance, *F*-test.
[c] Exact chi-square on two-way table.
[d] Non-parametric test of equality of medians.

oseltamivir-sensitive influenza H1N1 reported prophylactic use of oseltamivir. Prior vaccination in our study population was very rare: 3 patients with oseltamivir-sensitive and 2 patients with influenza B had been vaccinated (Table 2). Surprisingly, only a minority of patients were in favor of future vaccination, although this must be balanced against the fact that a large proportion of patients were children, and vaccination in children is currently not recommended in Luxembourg. Attack rates in households were similar between oseltamivir-resistant and oseltamivir-sensitive influenza H1N1 strains and influenza B.

Fig. 2 shows that partial NA gene sequences of oseltamivir-resistant strains differed by a minimum of 2 nucleotides from

Fig. 2. Phylogeny of partial NA gene sequences (795 nucleotides encoding the NA protein C-terminus) of a representative sample of influenza A H1N1 strains collected during the 2007/2008 seasonal epidemic in Luxembourg and other countries. Sequences from Luxembourg are in bold format and numbers of strains with identical sequences are given in brackets. All oseltamivir-resistant strains are highlighted (●). The tree was calculated with the Neighbour-Joining method (*p*-distance) using MEGA 4 software. The Influenza A H1N1 strain A/New Caledonia/20/1999 was used as an outgroup. All sequences from Luxembourg can be retrieved in Genbank under accession numbers FM174405–FM174468.

those of sensitive strains. One of the latter had the H275Y amino acid exchange, which induces the resistant phenotype. The second mutation is non-silent (D354G), and this same mutation was also found on the large majority of oseltamivir-resistant strains emerging worldwide in 2007/2008 (Meijer et al., 2009). NA gene sequences from oseltamivir-resistant strains circulating in Luxembourg did not form a separate cluster as compared with resistant strains from other countries. Thus the unexpectedly high frequency of oseltamivir-resistant influenza A H1N1 in Luxembourg was most probably correlated with the widespread circulation of such viruses in Europe.

From the data of our Laboratory confirmed influenza outpatients, we found little evidence that newly emerging oseltamivir-resistant influenza A H1N1 strains in 2007/2008 were any different from other circulating influenza strains in terms of patient characteristics, clinical picture or epidemiology. Following initial seeding in Luxembourg at the start of the season, oseltamivir-resistant strains appear to have spread at a similar rate as oseltamivir-sensitive strains, i.e. antiviral drug resistance did not seem to affect fitness. Thus, our results concur to a large extent with previous reports from different parts of the world [Europe (Meijer et al., 2009; Hauge et al., 2009), South Africa (Besselaar et al., 2008) and the United States (Dharan et al., 2009)]. However, the determinants of the relative frequency of oseltamivir resistance occurring in other European countries remain unclear and intriguing. Finally, the data presented with this study will provide an invaluable baseline for the assessing the severity of the pandemic novel A H1N1 strain spreading across the globe (Fraser et al., 2009; Lipsitch et al., 2009).

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