The Viral Etiology of an Influenza-like Illness During the 2009 Pandemic

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Many viruses are known to cause influenza-like illness (ILI); however, in nearly 50% of patients, the etiologic agent remains unknown. The distribution of viruses in patients with ILI was investigated during the 2009 A/H1N1 influenza pandemic (A/H1N1p). From June 2009 to January 2010, 660 patients with suspected influenza were questioned and examined, and nasal swabs were collected. All patient samples were tested for influenza virus, and 286 negative nasal swabs were tested further for 18 other respiratory viruses using real-time RT-PCR. Two waves of ILI were observed in the epidemic curve (weeks 35–42 and 42–49). At least eight viruses co-circulated during this period: human rhinovirus (HRV) (58), parainfluenza 1–4 viruses (PIV) (9), human Coronavirus (hCoV) OC43 (9), enterovirus (5), adenovirus (AdV) (4), and human metapneumovirus (hMPV) (2); however, 204 samples remained negative for all viruses tested. ILI symptoms, according to the Centers for Disease Control and Prevention criteria for ILI definition, were reported in 75% of cases. These patients had positive swabs for A/H1N1p, HRV, hCoV-OC43, PIV, AdV, and hMPV without significant difference with non-ILI patients. This study found that many respiratory viruses circulated during this period and that the A/H1N1p did not impact on the kinetics of other respiratory viruses. The proportion of non-documented cases remains high. ILI could not distinguish A/H1N1p infection from that due to other respiratory viruses. However, in multivariate analysis, cough, chills, hypothermia, and dyspnea were associated significantly with influenza virus versus other respiratory viruses. J. Med. Virol. 84:1071–1079, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: respiratory viruses; influenza A virus, A/H1N1p subtype; rhinovirus; influenza-like-illness; acute respiratory tract infection

INTRODUCTION

Acute respiratory infection is one of the leading causes of child and adult morbidity and mortality throughout the world [Williams et al., 2002]. Determining the etiological diagnoses of patients who have respiratory symptoms remains a challenge both in the clinic and laboratory. Differentiating infections caused by influenza viruses from those caused by other respiratory viruses is essential for case management, as illustrated during the 2009 A/H1N1 influenza pandemic (A/H1N1p). Many definitions of influenza-like illness (ILI) have been used worldwide in influenza surveillance; however, the sensitivity and positive predictive value of such definitions significantly vary depending on the co-circulation of other respiratory viruses in the community [Boivin et al., 2000; Lee et al., 2011; Thursky et al., 2003]. The identification of the respiratory viruses that are responsible for influenza-like illness has been reported in many countries, and the percentage of positive swabs for at least one virus ranges from 32% to 65% [Bellei et al., 2008; Laguna-Torres et al., 2009; Ren et al., 2009; Buecher

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et al., 2010; Renois et al., 2010; Razanajatovo et al., 2011). Influenza-like illness can be attributed to a wide range of respiratory viruses, including influenza viruses, adenoviruses (AdV), respiratory syncytial virus (RSV), enteroviruses (EVs), human rhinovirus (HRV), and parainfluenza viruses (PiVs) [Bellei et al., 2008; Laguna-Torres et al., 2009; Ren et al., 2009; Buecher et al., 2010; Renois et al., 2010; Razanajatovo et al., 2011]. Recently, several viruses have been associated with respiratory infections, including human metapneumovirus (hMPV) [van den Hoogen et al., 2001], human coronavirus NL63 (HCoV-NL63) [van der Hoek et al., 2004], human coronavirus HKU1 (HCoVHKU1) [Woo et al., 2005], as well as human bocavirus (HBoV) [Allander et al., 2005]. Three novel polyomaviruses, KIPyV, WUPyV, and MCPyV, have been detected recently in the respiratory tracts of humans; however, their pathogenicity remains controversial [Norja et al., 2007; Babakir-Mina et al., 2011].

The 2009 A/H1N1p influenza pandemic provided a unique opportunity to investigate the distribution of different viruses in patients with influenza-like illness in a large sample of the general population. Few studies have described epidemiological and clinical data for different respiratory viruses that were identified to be circulating during the A/H1N1p pandemic [Hombrouck et al., 2011; Lee et al., 2011; Raboni et al., 2011; Smit et al., 2011a,b]. This study describes the prevalence of 19 viruses in patients suspected with influenza by the general practitioner and then sent to a referral center for nasal swab sampling and subsequent laboratory testing during the A/H1N1p outbreak.

**MATERIALS AND METHODS**

**Respiratory Specimens**

From June 2009 to January 2010, patients presenting with influenza-like illness or suspected influenza were either referred to by their general practitioner, or consulted directly the doctor’s group set up specifically for the management of suspect patients during the 2009 influenza pandemic at the Infectious Disease and Tropical Medicine Department of the North Hospital, Marseille, France.

Upon admission, patients were questioned and examined, and nasal swabs were collected and tested at the point-of-care (POC) laboratory by a rapid influenza diagnostic test (RIDT) and real-time RT-PCR (rRT-PCR) [Ninove et al., 2010; Nougairede et al., 2010]. After obtaining oral consent, epidemiological and clinical questionnaires were completed while the patients waited for the RIDT result to be sent back by the POC lab. Patients with negative RIDT results returned home with isolation measures recommended until the result of the rRT-PCR assay was obtained. When the rRT-PCR results were obtained 12 hr later, patients with positive samples were contacted by telephone, and those with co-morbidity risk factors were proposed for hospitalization, oseltamivir therapy, and isolation measures. For those without co-morbidity risk factors, only symptomatic treatment was recommended.

**Detection of Respiratory Viruses**

RIDT was performed using the Directigen EZ influenza A + B test (BD EZ Flu A + B, Becton, Dickinson) according to the manufacturer’s instructions. RNA extraction: 200 μl of the respiratory sample prepared for RIDT were spiked with 10 μl of in-house MS2/T4 phages internal control [Ninove et al., 2011]. RNA was extracted and eluted in 90 μl using the BioRobot EZ1 Workstation and the EZ1 Virus Mini Kit v2.0 (Qiagen, Courtaboeuf, France).

Reverse transcription was performed with the Taqman Reverse Transcription kit (Applied Biosystems, Branchburg, NJ) with 20 μl of RNA, 22 μl of MgCl2, 10 μl of 10× buffer, 20 μl of 10 mM dNTPs, 5 μl of hexamers (at 1/10 dilution), 2.5 μl of Multi-scribe, and 2 μl of RNase inhibitor in a 100 μl final volume. The cycling program was 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. For each sample, two reverse transcriptions in a 100 μl final volume were done, resulting in a 200 μl volume of cDNA to be used in PCR tests.

PCR assays were performed using the qPCR Mastermix-No Rox kit (Eurogentec, Angers, France) with 10 μl of cDNA, 25 μl of Mastermix, 1 μl of each primer (10 μM), and 0.4 μl of probe (10 μM) in combination with a Stratagene MX3005P QPCR system (Agilent Technologies, La Jolla, CA). The primers and probes that were used in this study are listed in Table I. The cycling program was conducted at 50°C for 2 min, 95°C for 10 min, 45 cycles at 95°C for 15 sec, and 60°C for 60 sec.

**Internal and External Controls**

All steps (extraction, RT, PCR) were monitored with our universal internal control assay based on the use of DNA and RNA bacteriophages as described previously [Ninove et al., 2011]. PCR detection of T4 and MS2 bacteriophages was performed in parallel with other PCR using the same cycling program in a 15-μl final volume with 3 μl of cDNA, 7.5 μl of Mastermix, 0.3 μl of each primer (10 mM), and 0.15 μl of probe (10 μM). For each sample, the run was validated by the results that were obtained for T4 and MS2 [Ninove et al., 2011].

**Statistical Analyses**

The questionnaire was entered anonymously into the database with Epidata 3.1 (Centers for Disease Control and Prevention criteria for influenza-like illness, CDC, Atlanta, GA), and data were analyzed with SPSS, version 19.0 (SPSS Inc., Chicago, IL). To identify the clinical characteristics of each group of patients, all potential variables were first assessed individually in a univariate model, and P values were
| Viral etiology | Sequence of primers and probes | Protocol reference |
|---------------|--------------------------------|-------------------|
| Influenza virus A | GAGCTAAGAGAGCAATTGAG | Duchamp et al. [2010] |
| Influenza virus H3N2 | CATYCTGGTGGATGAGGCCCAT | van Elden et al. [2001] |
| Rhinovirus | CGTGCTGTTTTCGCTGTTGCA-TAMRA | Lu et al. [2008] |
| Metapneumovirus | CATATAAGCATGCTATATTAAAAGTCTC | Mackay et al. [2003] |
| Respiratory syncytial virus A | GCA CAT AAT TAG GAG GTT CAA A | van Elden et al. [2003] |
| Respiratory syncytial virus B | TGATAATCGACCTTCTTATATTTAAGTGG | van Elden et al. [2003] |
| Human coronavirus 229E | AAA GGG CTA TAA AAG AA TAA GTT ATT CT | van Elden et al. [2004] |
| Human coronavirus OC43 | CCT GCC TGA CCA CCA ATG GGT TGA TAG GT | van Elden et al. [2004] |
| Human coronavirus NL63 | CAG GGC TCA AAG GCA ATG GCC GGC GGT TTT TGG | Tiveljung-Lindell et al. [2009] |
| Human coronavirus KU1 | CAC TCT TAT CTC CTA CGA TGT TTC | Tiveljung-Lindell et al. [2009] |
| Enterovirus | GCC TCT GAT GAA ATT TTC AAG TGC TAC TTA GA | Watkins-Riedel et al. [2002] |
| Parechovirus | GTG ATG TTT TAT AAC TAC TGA TCT TGC TGG | Benshop et al. [2008] |
| Polyomavirus KI/KU | TTCGAGATGAAATGAGCAGTT | Lindau et al. [2009] |
| Parainfluenza virus 1/2/3/4 | ACA GAT GAA ATT TCC TTA CGA ATG TAC TTA AGT | Tong et al. [2008] |
| Bocavirus | AGA GGC TCG GCC TCA TAT CA | Allander et al. [2007] |
| Adenovirus | GCC AGC GTG GGG TTT CTA AAC TT | Heim et al. [2003] |

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measured for qualitative variables using Pearson’s chi-square test or Fisher’s exact test and for continuous variables using the Mann–Whitney non-parametric test. Correlations were assessed using the Spearman non-parametric test. Variables with P values <0.20 were retained and entered into a multivariate logistic regression analysis.

RESULTS

During the 29 weeks of the outbreak, 660 patients were seen at the outpatient clinic. Two peaks of influenza-like illness were detected, the first peak (181 patients) from week 36 to 41 (wave 1) and the second peak (256 patients) from week 43 to 48 (wave 2) (Fig. 1). Among the 660 patients, 59.8% were female, more than half (53.9%) were between the ages of 20 and 40 and 6 (0.9%) were older than 65. The clinical characteristics and risk factors for serious illness are listed in Table II. The study was authorized by the ethics committee board of the university, number 10-0010.

Virus Detection

Among the 660 patients, 158 were positive for A/H1N1p. Among the 502 patients with negative rRT-PCR results for A/H1N1p, 286 samples (randomly chosen from the samples still available, 104 patients were seen during the wave 1, 123 patients were seen during the wave 2, and the remaining 59 patients during the other periods) were tested for 18 other respiratory viruses: 82 were positive for at least one virus (58 were positive for HRV, nine for HCoV OC43, five for EV, five for PIV1, one for PIV2, three for PIV4, four for AdV, and two for hMPV). The remaining 204 samples were found negative for all viruses tested in the study (Fig. 2).

During the first wave, 15 (8.3%) patients had positive swabs for A/H1N1p and among the 104 patients who were tested for other viruses, 25 (24%) had positive swabs for HRV and 72 (69%) had negative swabs. During the second wave, the percentage of patients who tested positive for A/H1N1p was significantly higher than that observed during the first wave (42.2%, \( P < 0.001 \), OR = 8, and 95% CI: 4.5; 14.5), whereas, among the 123 patients who were tested for other viruses, HRV had a consistent prevalence (16.3%, \( P = 0.143 \)). Among the 286 samples tested for other viruses, co-infection was identified in five patients: one HRV with ADV, one HRV with PIV4, one EV with hCoV OC43, one hMPV with ADV, and one PIV with ADV. No patients with co-infections were hospitalized.

Clinical Features

Patient characteristics were stratified by infection status (Table II). In 75% of the cases, patients...
TABLE II. Comparative Clinical and Epidemiological Characteristics of all of the Patients Stratified by A/H1N1p and HRV Infection

| Characteristics               | N (%) total | N (%) A/H1N1p | N (%) HRV | A/H1N1p vs. non-A/H1N1p | Odds ratio (95% CI) | P-value | HRV vs. non-HRV | Odds ratio (95% CI) | P-value | A/H1N1p vs. HRV | Odds ratio (95% CI) | P-value |
|------------------------------|-------------|---------------|-----------|--------------------------|---------------------|---------|----------------|---------------------|---------|----------------|---------------------|---------|
| Sex                          |             |               |           |                          |                     |         |                |                     |         |                |                     |         |
| Female                       | 395 (59.8)  | 79 (50)       | 19 (32.8) | <0.01                    | 1.7 (1.19; 2.44)    | 0.6      |                |                     |         |                |                     |         |
| Male                         | 265 (40.2)  | 79 (50)       | 39 (67.2) | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| Age                          |             |               |           |                          |                     |         |                |                     |         |                |                     |         |
| Mean (SD)                    | 31 (14.27)  | 26.6 (12.12)  | 32.6 (12.7) | <0.0001                  | 0.8                 |         |                |                     |         |                |                     |         |
| 11–20                        | 110 (16.7)  | 40 (25.5)     | 10 (17.2) | <0.0001                  | 1.0                 |         |                |                     |         |                |                     |         |
| 21–30                        | 195 (29.5)  | 56 (35.7)     | 17 (29.3) | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| 31–40                        | 160 (24.2)  | 35 (22.3)     | 14 (24.1) | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| 41–50                        | 78 (11.8)   | 7 (4.5)       | 9 (15.5)  | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| 51+                          | 76 (11.5)   | 9 (5.7)       | 7 (12.1)  | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| Clinical characteristics     |             |               |           |                          |                     |         |                |                     |         |                |                     |         |
|         |             | Influenza-like illness | | | | | | | | | | |
| Fever | 556 (84.9)  | 143 (90.5)    | 46 (79.3) | <0.05                    | 1.9 (1.08; 3.47)    | 0.5      |                |                     |         |                |                     |         |
| Cough | 545 (83.2)  | 153 (96.8)    | 50 (86.2) | <0.0001                  | 8.2 (3.28; 20.49)   | 0.5      |                |                     |         |                |                     |         |
| Asthenia | 611 (93.3)  | 151 (95.6)    | 51 (87.9) | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| Myalgia | 521 (79.7)  | 126 (80.3)    | 43 (74.1) | <0.05                    | 0.5 (0.26; 0.92)    |          |                |                     |         |                |                     |         |
| Rhinorrhea | 414 (63.2)  | 117 (74.1)    | 47 (78.1) | <0.05                    | 1.9 (1.29; 2.86)    | <0.001   | 3.3 (1.61; 6.62) | 0.3      | <0.05 | 0.4 (0.18; 0.92) |                     |         |
| Headache | 507 (77.4)  | 123 (78.7)    | 40 (69)   | <0.05                    | 0.6 (0.29; 1.06)    | 0.2      |                |                     |         |                |                     |         |
| Arthralgia | 259 (39.7)  | 65 (41.4)     | 18 (31)   | <0.05                    | 0.6 (0.29; 1.06)    |          |                |                     |         |                |                     |         |
| Diarrhea | 109 (16.6)  | 29 (18.4)     | 7 (12.1)  | <0.05                    | 0.6 (0.29; 1.06)    |          |                |                     |         |                |                     |         |
| Nausea | 230 (35.2)  | 62 (39.2)     | 13 (22.8) | <0.05                    | 0.5 (0.24; 0.93)    | <0.05    | 0.5 (0.23; 0.92) | 0.2      | <0.05 | 0.5 (0.23; 0.92) |                     |         |
| Vomiting | 104 (15.9)  | 35 (22.2)     | 8 (14)    | <0.05                    | 0.5 (0.24; 0.93)    | <0.05    | 0.5 (0.23; 0.92) | 0.2      | <0.05 | 0.5 (0.23; 0.92) |                     |         |
| Conjunctive | 66 (11.1)   | 23 (17.8)     | 6 (12)    | <0.05                    | 2.1 (1.24; 3.71)    | 1.0      |                |                     |         |                |                     |         |
| Medical history/                           | | | | | | | | | | | | |
|         |             |             |           |                          |                     |         |                |                     |         |                |                     |         |
| Recent travel | 106 (16.2)  | 18 (11.4)     | 8 (13.8)  | 0.06                    | 0.6 (0.35; 1.03)    | 0.6      |                |                     |         |                |                     |         |
| Live locally | 658 (99.7)  | 158 (100)    | 57 (98.3) | 1.0                     | 0.4                 | 0.3      |                |                     |         |                |                     |         |
| Pregnant (2nd trimester) | 36 (5.6)    | 6 (4)        | 7 (12.3)  | 0.06                    | 2.7 (1.01; 7.41)    | <0.05    | 3.4 (1.08; 10.5) | 0.3      | <0.05 | 0.5 (0.26; 0.92) |                     |         |
| Chronic bronchopathy | 92 (14.4)   | 21 (14.1)     | 13 (22.8) | 0.9                     | 0.1                 | 0.1      |                |                     |         |                |                     |         |
| Cardiopathy | 11 (1.7)    | 3 (2)        | 1 (1.8)   | 0.7                     | 1.0                 | 1.0      |                |                     |         |                |                     |         |
| Neurologic | 10 (1.6)    | 5 (3.4)      | 1 (1.8)   | 0.05                    | 3.4 (0.97; 11.88)   | 0.4      |                |                     |         |                |                     |         |
| Hematologic | 7 (1.1)     | 2 (1.3)      | 0 (0)     | 0.7                     | 0.6                 | 1.0      |                |                     |         |                |                     |         |
| Metabolic | 38 (5.9)    | 12 (8.1)     | 2 (3.5)   | 0.2                     | 0.7                 | 0.4      |                |                     |         |                |                     |         |
| Immunodeficiency | 11 (1.7)    | 2 (1.3)      | 2 (3.5)   | 1.0                     | 0.3                 | 0.5      |                |                     |         |                |                     |         |
| Obesity | 9 (1.4)     | 0 (0)        | 0 (0)     | 0.1                     | 0.6                 |           |                |                     |         |                |                     |         |
| Alcohol/hepatopathy | 9 (1.4)     | 3 (2)        | 0 (0)     | 0.4                     | 0.6                 |           |                |                     |         |                |                     |         |
| >65 Years old | 60 (9.0)    | 1 (0.7)      | 0 (0)     | 1.0                     | 0.6                 | 1.0      |                |                     |         |                |                     |         |
| Risk factor (one or more) | 189 (28.6)  | 44 (27.8)    | 21 (36.2) | 0.8                     | 0.6                 | 1.0      |                |                     |         |                |                     |         |
| Hospitalization | 22 (3.3)    | 5 (3.2)      | 5 (8.3)   | 0.9                     | 0.7                 | 2.3      |                |                     |         |                |                     |         |
| Total | 660 | 186 | 58 |

HRV, rhinovirus; A/H1N1p, 2009 pandemic A/H1N1 influenza virus.
Presented with an influenza-like illness according to the definition proposed by the CDC with the following symptoms: a temperature >37.8°C and either a cough or sore throat [Babcock et al., 2006; CDC, 2010]. Only three patients were asymptomatic. Among the influenza-like illness patients, 100 (27.9%) were positive for A/H1N1p and 27 (18.1%), 6 (4%), 5 (3.4%), 3 (2.1%), and 1 (0.7%) of the tested patients, were positive for HRV, PIV (1, 2, or 4), hCoV OC43, ADV, and hMPV, respectively (Table III). Of all of the patients, 22 were hospitalized later, 5 (22.7%) had confirmed A/H1N1p infection, and 2 (9%) tested positive for HRV.

Patients who tested positive for A/H1N1p were significantly younger (26.61 years with 95% CI: 25.61–27.61) compared to those who did not have A/H1N1p infection (32.56 years with 95% CI: 31.56–33.56).

**TABLE III. Etiologic Agent of Viral Respiratory Infection in Patients With or Without Influenza-like Illness (According to the CDC’s Definition) From June 2009 to January 2010 in Marseille, France**

| Virus                  | Clinical presentation | P-value  |
|------------------------|-----------------------|----------|
|                        | Influenza-like illness | Non-influenza-like illness |
| A/H1N1p                | 100 (27.9)            | 30 (25.4) | 0.596 |
| EV                     | 1 (0.3)               | 1 (0.8)  | 0.406 |
| HRV                    | 27 (7.5)              | 7 (5.9)  | 0.537 |
| hMPV                   | 1 (0.3)               | 0        | 0.565 |
| hCoV OC43              | 5 (1.4)               | 1 (0.8)  | 0.641 |
| PIV 1                  | 4 (1.1)               | 1 (0.8)  | 0.803 |
| PIV 2                  | 0                     | 0        | 0.803 |
| PIV 4                  | 2 (0.6)               | 1 (0.8)  | 0.728 |
| AdV                    | 3 (0.8)               | 0        | 0.315 |
| Negative for all viruses tested | 108 (30.2) | 39 (33.1) | 0.311 |
| Not tested for respiratory viruses | 109 (30.4) | 39 (33.1) | 0.596 |
| Total                  | 358                   | 118      |        |

ADV, adenovirus; hCoV OC43, human coronavirus OC43; EV, enterovirus; hMPV, human metapneumovirus; HRV, rhinovirus; A/H1N1p, 2009 pandemic A/H1N1 influenza virus; PIV, parainfluenza virus.

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24.70–28.52) than those who tested negative for A/H1N1p (32.28 years with 95% CI: 30.91–33.67; \( P = 0.0001 \)). When analyzed by univariate analysis, cough (96.8%), self-reported fever (90.5%), rhinorrhea (74%), chills (74%), vomiting (22.1%), conjunctive hyperemia (17.8%), and dyspnea (34.1%) were significantly more prevalent in A/H1N1p-positive patients with odds ratios (OR) ranging from 1.72 to 8.20 significantly more prevalent in A/H1N1p-positive patients.

Some EVs remained undetected, five samples were tested positive for positive EVs (1.7% of tested patients), which is consistent with previous studies in patients with acute respiratory infection or influenza-like illness. Nine patients were positive for at least one of the several viruses. Although PIV1, PIV2, and PIV3 are considered to be the most frequently identified PIVs, PIV1, and PIV4 were detected primarily, which is similar to the findings of Renois et al. [2010].

The real-time PCR method used for the diagnosis of EV was designed most specifically for diagnostics of central nervous system infections; however, recent studies have used a generic pan-EV/rhinovirus real-time PCR and have identified a novel respiratory EV that could not be detected by the systems designed for meningitis diagnostics [Watkins-Riedel et al., 2002; Tapparel et al., 2009]. Although it is possible that some EVs remained undetected, five samples were tested positive for positive EVs (1.7% of tested patients), which is consistent with previous studies in patients with acute respiratory infection or influenza-like illness.

In this study, four samples (1.4%) were positive for AdV and two samples (0.7%) were positive for hMpV. These prevalences are comparable with previous reports [Bellei et al., 2008; Laguna-Torres et al., 2009; Ren et al., 2009].

The absence of RSV infection could be explained by the mean age (31 year old) of the tested population, and by the delayed epidemic of RSV infection in France during the 2009–2010 winter season [Casalegno et al., 2009a,b]. The clinical significance of co-infections is unclear [Jartti et al., 2004]. In this study, only 5 of the 286 tested samples (1.7%) were positive for more than one respiratory virus, which was lower than previously reported [Esper et al., 2011; Hombrouck et al., 2011; Raboni et al., 2011; Tokarz et al., 2011], whereas this result is biased by the fact that only the patients who were negative for A/H1N1p were tested for the other viruses. During the A/H1N1p pandemic, circulation of influenza B and A/H3N2 was null and very limited, respectively [Renois et al., 2010; Nakamura et al., 2011; Tokarz et al., 2011]. Therefore, patients who had negative RIDT results were not tested for influenza B.

There are multiple clinical definitions of influenza-like illness. None are satisfactorily sensitive and specific for defining influenza virus infection [Thursky et al., 2003; CDC, 2010]. Although 75% of the clinical presentations were defined as influenza-like illness according to the CDC definition [CDC, 2010], only 28% were confirmed influenza by laboratory documentation. Moreover, the percentage of patients with A/H1N1p-positive swabs did not differ significantly between patients with symptoms of influenza-like illness and those who did not present with these symptoms. Patients with influenza-like illness also had swabs that tested positive for HRV, EV, hMpV, AdV, PIVs, or hCoV OC43. The clinical characteristics of patients.
with A/H1N1p infection have been reported in several countries [Crum-Cianfone et al., 2009; Ong et al., 2009; Kim et al., 2010; Hombrouck et al., 2011; Lee et al., 2011; Smit et al., 2011a,b]. Of these eight studies, the clinical features that were associated mostly with A/H1N1p were the cough and the fever.

Several studies have reported the results from respiratory virus testing using respiratory samples that were obtained from patients with influenza-like illness or acute respiratory infection throughout the world and during different times. Although the target populations, inclusion criteria, seasonality, climate, environment, diagnostic methods, and numbers of viruses or bacteria that were tested differed, the proportion of non-documented cases remained relatively high [Bellei et al., 2008; Laguna-Torres et al., 2009; Renois et al., 2010].

In conclusion, this study found (i) that many respiratory viruses circulated during the A/H1N1p pandemic in France, (ii) that A/H1N1p virus circulation did not impact on the kinetics of other respiratory viruses, (iii) that the percentage of non-documented cases remains high and therefore justify to pursue technical development and to enlarge the variety of microorganisms in detection panels, (iv) that CDC definition of influenza-like illness symptoms are not capable to distinguish A/H1N1p virus from other respiratory viruses, and finally, and (v) that the most specific criteria in favor of A/H1N1p infection was cough.

Systematic testing for respiratory viruses is necessary to improve the targeting of appropriate antiviral treatments. Specific studies exploring (i) the prevalence of coinfections and their clinical characteristics, (ii) socio-economic consequences of the different microorganisms involves in respiratory infections not only at the hospital level but more broadly inside and outside of the hospital are necessary for a better management of cases.

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