



Clinical characteristics and outcomes of influenza and other influenza-like illnesses in Mexico City

Arturo Galindo-Fraga^{a,*}, Ana A. Ortiz-Hernández^b, Alejandra Ramírez-Venegas^c, Rafael Valdez Vázquez^d, Sarbelio Moreno-Espinosa^e, Beatriz Llamosas-Gallardo^b, Santiago Pérez-Patrigeon^a, Maggie Salinger^f, Laura Freimanis^g, Chiung-yu Huang^f, Wenjuan Gu^h, M. Lourdes Guerrero^a, John Beigel^h, Guillermo M. Ruiz-Palacios^a, for The La Red ILI 002 Study Group

^a Instituto Nacional de Ciencias de Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga 15, Col. Sección XVI, Mexico City, DF 14000, Mexico

^b Instituto Nacional de Pediatría, Mexico City, Mexico

^c Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico

^d Hospital General "Dr. Manuel Gea González", Mexico City, Mexico

^e Hospital Infantil de México "Dr. Federico Gómez", Mexico City, Mexico

^f National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

^g Westat, Rockville, Maryland, USA

^h SAIC Frederick in support of National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

ARTICLE INFO

Article history:

Received 19 September 2012

Received in revised form 28 December 2012

Accepted 6 January 2013

Corresponding Editor: Eskild Petersen, Skejby, Denmark

Keywords:

Respiratory viral pathogens

Epidemiology

Hospital burden of disease

Influenza

Respiratory syncytial virus

Rhinovirus

Coronavirus

SUMMARY

Background: Influenza-like illnesses (ILI) are estimated to cause millions of deaths annually. Despite this disease burden, the etiologic causes of ILI are poorly described for many geographical regions.

Methods: Beginning in April 2010, we conducted an observational cohort study at five hospitals in Mexico City, enrolling subjects who met the criteria for ILI. Evaluations were conducted at enrollment and on day 28, with the collection of clinical data and a nasopharyngeal swab (or nasal aspirate in children). Swabs were tested by multiplex PCR for 15 viral pathogens and real-time PCR for influenza.

Results: During the first year, 1065 subjects were enrolled in this study, 55% of whom were hospitalized; 24% of all subjects were children. One or more pathogens were detected by PCR in 64% of subjects, most commonly rhinovirus (25% of all isolates) and influenza (24% of isolates). Six percent of subjects died, and of those, 54% had no pathogen identified. Rhinovirus was the most common pathogen among those who died, although it did not have the highest case fatality rate.

Conclusions: Multiple respiratory viruses beyond influenza are associated with significant morbidity and mortality among adults and children in Mexico City. Detection of these agents could be useful for the adjustment of antibiotic treatment in severe cases.

© 2013 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

In April 2009, an outbreak of respiratory disease in young people was reported in Mexico City,¹ and the etiologic agent was identified as influenza A virus H1N1pdm09.² This outbreak and its consequences compelled the Ministry of Health of Mexico and the US National Institute of Allergy and Infectious Diseases (NIAID) to collaborate in establishing a Mexican Emerging Infectious Diseases Clinical Research Network (La Red).

Acute respiratory infections are estimated to cause 3.9 million deaths annually,³ many of which are influenza-like illnesses (ILI).

The etiology of ILI in Mexican individuals seeking medical care is not well-defined. It has been reported in other populations that multiple respiratory viruses such as parainfluenza virus, rhinovirus, adenovirus, metapneumovirus, respiratory syncytial virus (RSV), and coronavirus can cause ILI,⁴ but the virologic etiology varies greatly among geographic locales, age groups, seasons, and years.^{5–13} Some studies have suggested that specific symptoms could be used for the clinical prediction of the etiology of ILI,^{14–18} though most of these studies only compared influenza to non-influenza etiology.

Although the outcomes of subjects infected with various influenza subtypes have been well documented,^{19–21} there is relatively little description of outcomes from other viral etiologies of ILI. Shlomai et al. compared outcomes of those hospitalized with an ILI, and found mortality of 4.6% in influenza patients vs. 7% in

* Corresponding author. Tel.: +52 55 5487 0900 ext. 2420; fax: +52 55 5513 0010.
E-mail address: galindofraga@yahoo.com (A. Galindo-Fraga).

influenza-negative ILI patients.¹⁸ As further demonstration of the burden that non-influenza etiological agents contribute to ILIs, Widmer et al. reported that RSV hospitalization rates exceeded those of influenza (15.01 and 11.81 per 10 000, respectively).²²

It is important to know the characteristics and seasonal behavior of the different types of virus causing ILI. Although current clinical diagnostic tests of all viruses are high in cost, improved diagnosis would allow a more judicious use of empirical wide-spectrum antibiotic treatment, which in many cases could be suspended, and thus reduce the cost of hospitalization.

Recognizing the need to investigate both influenza and non-influenza ILIs in the Mexican population, La Red implemented a study with the objective of describing the etiology, symptoms, and outcomes of subjects presenting with ILI in Mexico City.

2. Methods

2.1. Study design and sites

Beginning in April 2010, this hospital-based observational cohort study was conducted at five hospitals in Mexico City. These hospitals are located in the south of Mexico City, and include a general hospital, a tertiary care hospital that serves mainly those with respiratory problems, a tertiary care hospital that serves the metabolic/surgical population, and two pediatric centers. These centers were chosen given their capacity to conduct clinical research.

2.2. Case definition and study population

The study population included subjects aged >3 months who presented with an ILI. ILI was defined by the presence of at least one respiratory symptom (e.g., shortness of breath, postnasal drip, and cough) and one of the two following criteria: (1) fever ($\geq 38^\circ\text{C}$ by any method) on examination, or participant-reported fever ($\geq 38^\circ\text{C}$), or feverishness in the past 24 h; (2) one or more non-respiratory symptoms (e.g., malaise, headache, myalgia, and chest pain). The subjects included were those who sought medical attention at our centers and agreed to participate in the study.

2.3. Study procedures

Demographic data were collected at enrollment. A nasopharyngeal swab (Copan, Brescia, Italy), or a nasal aspirate in children, was obtained for PCR detection of respiratory pathogens and

sample storage. The samples were placed in transport medium, maintained at 4°C , and sent within 72 h to a central facility (Molecular Biology Laboratory, Infectious Diseases Department, INNSZ, Mexico City), where they were stored at -70°C . Clinical laboratory tests were carried out at enrollment. If available, the results of tests taken for standard clinical care (chest X-ray, bacteriological cultures, arterial blood gases, and liver tests) were also abstracted from the medical records. Subjects were seen again on day 28, and follow-up information was also obtained by phone on days 14 and 60. At each visit, clinical information (symptoms, chronic medical conditions, previous treatment, impact on daily function, hospitalizations, and death) was assessed.

2.4. Virology

All samples were tested by real-time reverse transcriptase PCR for influenza A following the Centers for Disease Control and Prevention (CDC) protocol (CDC 2009), as described previously.²³

For multi-pathogen detection, the samples were tested with the RespiFinder19 kit (previously RespiFinder Plus, from PathoFinder BV, Maastricht, The Netherlands), as described previously.²⁴ This multiplex PCR test can detect and differentiate 15 viruses (coronavirus NL63, OC43, and 229E, human metapneumovirus, influenza A, influenza A H5N1, influenza B, parainfluenza virus types 1 to 4, RSV A and B, rhinovirus, and adenovirus), as well as four bacteria (*Bordetella pertussis*, *Chlamydomphila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*). The analytical sensitivity (reported by the manufacturer) varies between 5 and 50 copies per reaction for most targets.²⁵

For the samples with discordant real-time reverse transcriptase PCR results and multiplex PCR for influenza, the samples were further tested with a third method, based on a final-point nested PCR protocol.²⁶ For these samples, the result of this test was considered the final result.

2.5. Statistical analysis

Data were analyzed with the Statistical Analysis System (SAS Institute, Cary, NC, USA). Median (range) and percentage were used to summarize quantitative and qualitative variables, respectively. To discard the role of chance, the Mann–Whitney test and the Chi-square or Fisher's exact test were used as appropriate. Univariate logistic regression analyses were performed to evaluate the effect of each baseline covariate on the risk of death. Odds ratios (OR) and the corresponding 95% confidence intervals (CI) were reported

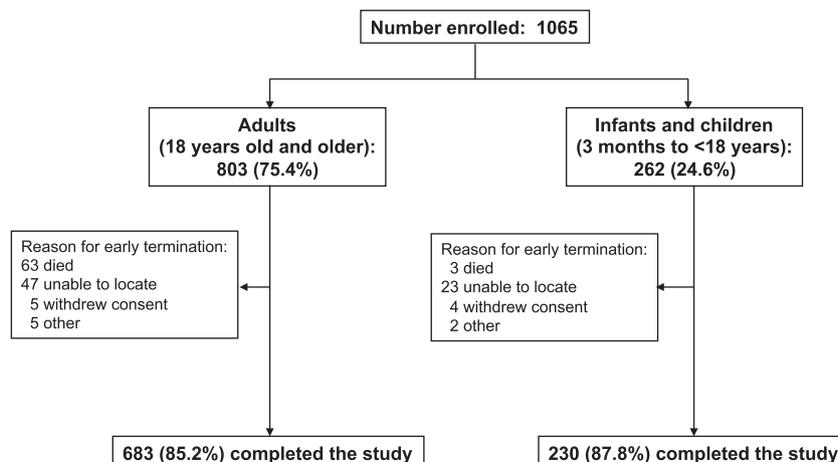


Figure 1. Enrollment and follow-up.

based on the fitted logistic regression models. All *p*-values were two-sided, with no correction for multiple comparisons; *p* < 0.05 was considered statistically significant.

3. Results

3.1. Patients

From April 11, 2010 through April 10, 2011, 1065 subjects were enrolled in this study. A total of 86 withdrew or were withdrawn from the study (Figure 1). Characteristics of the study participants are listed in Table 1. Just under a quarter of the study subjects were children and 58.8% were female. Four hundred thirty-six (40.9%) subjects were hospitalized. Asthma and cardiovascular disease were the most common chronic medical conditions, though neither condition accounted for more than 17% of subjects.

3.2. Etiology

A total of 821 etiologic agents were identified among the 678 (64% of total) subjects from whom a pathogen was isolated. Rhinovirus was the most frequently isolated pathogen, infecting 15.3% of enrolled subjects and accounting for 25.3% of all isolates. Rhinovirus was followed by influenza, which was detected in 14.3% of subjects, and 24% of isolates. Adenovirus, coronavirus, metapneumovirus, and RSV all had an isolate frequency of 9% or greater. No pathogen was detected for 35.5% of subjects (Table 2).

Among the 125 subjects (11.9%) with mixed virus infections, 62 unique combinations were recorded. The most common combinations were adenovirus/rhinovirus in nine subjects (7.2% of combinations), adenovirus/metapneumovirus in eight subjects (6.4%), and influenza B/metapneumovirus in eight subjects (6.4%).

Influenza was the most common virus involved in co-infections (when considering all subtypes), present in 45/125 (36%) combinations. Most combinations were limited to two viruses.

3.3. Seasonal distribution of viruses

The four most common pathogens (rhinovirus, influenza, coronavirus, and RSV) all had seasonal distributions with more isolates observed during cooler months (December–March). The seasonal variation of rhinovirus and RSV, however, was less pronounced than that of coronavirus and influenza (Figure 2). During April – November, rhinovirus accounted for 20–60% of all ILI. However, between December and March – a period during which the total number of ILI cases increased – the relative contribution of rhinovirus to ILI was only 10–20%.

3.4. Vaccination

Previous influenza vaccination was reported for 46.2% of the children and 47.7% of the adults. We found no difference in the proportion of vaccinated and unvaccinated subjects in the persons with virus or without virus detection, neither for individual agents nor as a group.

3.5. Symptoms

Evaluation across the four most common pathogens showed that children were more likely to have fever, productive cough, and nausea, whereas adults were more likely to have fatigue, headache, and sore throat (Table 3). Respiratory symptoms, such as dyspnea, were more common among those with rhinovirus or RSV, and gastrointestinal symptoms, such as nausea or diarrhea, were more common among those with influenza.

Table 1
Baseline characteristics

Demographics	Total, N=1065	Children, n=262	Adults, n=803
Age, years			
Median	33		
Range	0–96		
≤18	262 (24.6%)		
19–59	614 (57.6%)		
≥60	189 (17.8%)		
Sex			
Male	439 (41.2%)	132 (50.4%)	307 (38.2%)
Female	626 (58.8%)	130 (49.6%)	496 (61.8%)
Pregnant	4		4
Ethnicity			
Mixed	1026 (96.4%)	252 (96.2%)	775 (96.5%)
White	33 (3.1%)	10 (3.8%)	23 (2.9%)
Other	5 (0.5%)		5 (0.6%)
Medical history			
Influenza vaccination	504 (47.3%)	121 (46.2%)	383 (47.7%)
Asthma	151 (14.2%)	16 (6.1%)	135 (16.8%)
CVD	147 (13.8%)	17 (6.5%)	130 (16.2%)
Diabetes	99 (9.3%)		99 (12.3%)
Immunodeficiency	48 (4.5%)	8 (3.1%)	40 (5.0%)
Renal disorder	42 (3.9%)	4 (1.5%)	38 (4.8%)
COPD	36 (3.4%)		36 (4.5%)
BMI category ^a			
Obese	207 (19.9%)	23 (9.2%)	184 (23.3%)
Overweight	313 (30.2%)	42 (16.1%)	273 (34.6%)
Normal	417 (40.2%)	121 (48.6%)	296 (37.5%)
Underweight	101 (9.7%)	65 (26.1%)	36 (4.6%)
Median days since symptoms onset	1		
Inpatient at baseline	436 (40.9%)	113 (43.1%)	323 (40.2%)
Outpatient at baseline	629 (59.1%)	149 (56.9%)	480 (59.8%)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; SD, standard deviation.

^a BMI categories: underweight = BMI < 18.5 kg/m² (or <1 SD for children); normal weight = 18.5–24.9 kg/m²; overweight = 25–29.9 kg/m² (>1SD for children); obese = BMI of ≥30 kg/m² (>2 SD for children).

Table 2
Frequency distribution of viral isolates

Pathogen	Age <18 ^a , n (%)	Age 18–59 ^a , n (%)	Age ≥60 ^a , n (%)	Total ^a , n (%)	Total ^b , n (%)
Adenovirus	17 (6.6)	15 (2.5)	3 (1.6)	35 (3.3)	74 (9.0)
<i>Bordetella pertussis</i>	2 (0.8)	3 (0.5)	0 (0)	5 (0.5)	9 (1.1)
Coronavirus	12 (4.7)	50 (8.2)	15 (8.2)	77 (7.3)	118 (14.4)
229E	0	11	2	13	22
NL63	2	8	1	11	18
OC43	10	31	12	53	78
Influenza	43 (16.7)	89 (14.6)	18 (9.8)	150 (14.3)	197 (24.0)
A	3	5	0	8	11
A H1N1 2009	0	5	1	6	7
A H3N2	16	46	11	73	91
B	24	33	6	63	88
Metapneumovirus	11 (4.3)	22 (3.6)	9 (4.9)	42 (4.0)	78 (9.5)
<i>Mycoplasma pneumoniae</i>	1 (0.4)	4 (0.7)	1 (0.5)	6 (0.6)	10 (1.2)
Parainfluenza virus	6 (2.3)	12 (1.9)	2 (1.1)	20 (1.9)	42 (5.1)
1	0	0	0	0	3
2	0	0	0	0	3
3	6	7	1	14	24
4	0	5	1	6	12
Rhinovirus	29 (11.2)	100 (16.4)	32 (17.4)	161 (15.3)	208 (25.3)
RSV	34 (13.2)	15 (2.5)	8 (4.3)	57 (5.4)	85 (10.3)
A	33	15	6	54	72
B	1	0	2	3	13
Mixed infections	51 (19.8)	60 (9.9)	14 (7.6)	125 (11.9)	
Negative cases	52 (20.2)	238 (39.1)	83 (45.1)	373 (35.5)	
Total	258 (100)	609 (100)	184 (100)	1051 (100) ^c	821 (100)

RSV, respiratory syncytial virus.

^a Frequency by subject. Infections with more than one virus are counted as a single category (mixed infection).

^b Frequency by isolates. Excludes negative cases.

^c Fourteen subjects did not have a baseline sample for virologic testing.

3.6. Outcomes

Hospitalization was required for 585 subjects (54.9%) (Table 4). A lower percentage of subjects infected with coronavirus and influenza were hospitalized (32% and 29%) than those infected with rhinovirus and RSV (51% and 56%, respectively) (Table 3). In the pediatric population, RSV infections had the highest rate of hospitalization (76.5%), as well as the highest absolute number of hospitalizations ($n = 26$, 24.4% of all hospitalized pediatric subjects). Mixed infections ($n = 25$, 20.7%), rhinovirus ($n = 17$, 14.0%), and influenza ($n = 11$, 9.1%) also contributed significantly to hospitalization in the pediatric cohort. Rhinovirus was also the most common pathogen isolated from adults and the elderly who were hospitalized, followed by mixed infections, influenza, and metapneumovirus.

Of the 1065 patients enrolled, 66 died (6.2%). Of the patients who died, 54% had no pathogen identified. Rhinovirus was the most common etiologic agent identified in subjects who died (37.9% of deaths with a pathogen identified, and 17.5% of all deaths). Only four pediatric subjects died: two with RSV, one with parainfluenza, and one with *Mycoplasma*. Subjects ≥60 years of age infected with coronavirus, influenza, metapneumovirus, rhinovirus, or RSV all had mortality rates exceeding 10%. The highest mortality rate (27.7%, $n = 23$) was seen in the cohort of patients ≥60 years old with no identified pathogen, followed by rhinovirus. Mixed viral infections did not have an appreciably larger rate of hospitalization or death.

Subjects who died were more likely to have elevated creatinine, lactate dehydrogenase, and C-reactive protein, lower lymphocyte counts, and higher neutrophil counts (Table 5). Subjects who died were more likely to have underlying medical conditions (except asthma). Subjects on systemic steroids prior to enrollment were also more likely to die. Headaches and sore throats were seen more commonly in non-fatal cases, whereas rales (representing lower respiratory tract disease) were more likely in fatal cases. A

productive cough did not seem to differentiate fatal from non-fatal cases.

4. Discussion

This study presents the most thorough description of the causes of ILI in Mexico City to date. Using a multiplex PCR test, one or more pathogens were identified in 65% of enrolled subjects. The most common pathogen identified in the study was rhinovirus (25% of all isolates), and influenza was the second most common pathogen (24% of all isolates). This is consistent with other series of adult ILI in various geographic regions, where rhinovirus has been reported to account for 25–50% of adult ILI isolates, and influenza for 14–23%.^{10,13,27,28} Coronavirus – most commonly OC43, but also NL63 and 2229E – was also a significant contributor to the burden of ILI (14% of all isolates). Recent studies have reported coronavirus to be the third to fifth most common pathogen.^{10,13}

Among children in this study, the most common pathogen was influenza, followed by RSV and rhinovirus. Similar significant contributions of RSV and influenza have been noted in prior studies.^{29–31} Rhinovirus, however, was rarely tested in previous studies. In one other prospective cohort study of children with lower respiratory tract infections, rhinovirus was reported as second only to RSV in causing hospitalizations.³² The frequency at which it was isolated in our hospitalized children (14%, 17/121) suggests that rhinovirus is a previously under-appreciated pathogen for this age group.

Rhinovirus was a cause of significant morbidity in all age groups of our study population, such that 60–80% of individuals infected with rhinovirus required hospitalization. Rhinovirus was also the most common isolate from subjects who died (isolated from 11/66 subjects). Given the study design, a clear attribution to the cause of hospitalization or death is not possible.

Coronavirus species made up 14% of all isolates in our population. Coronavirus can present with a similar syndrome to

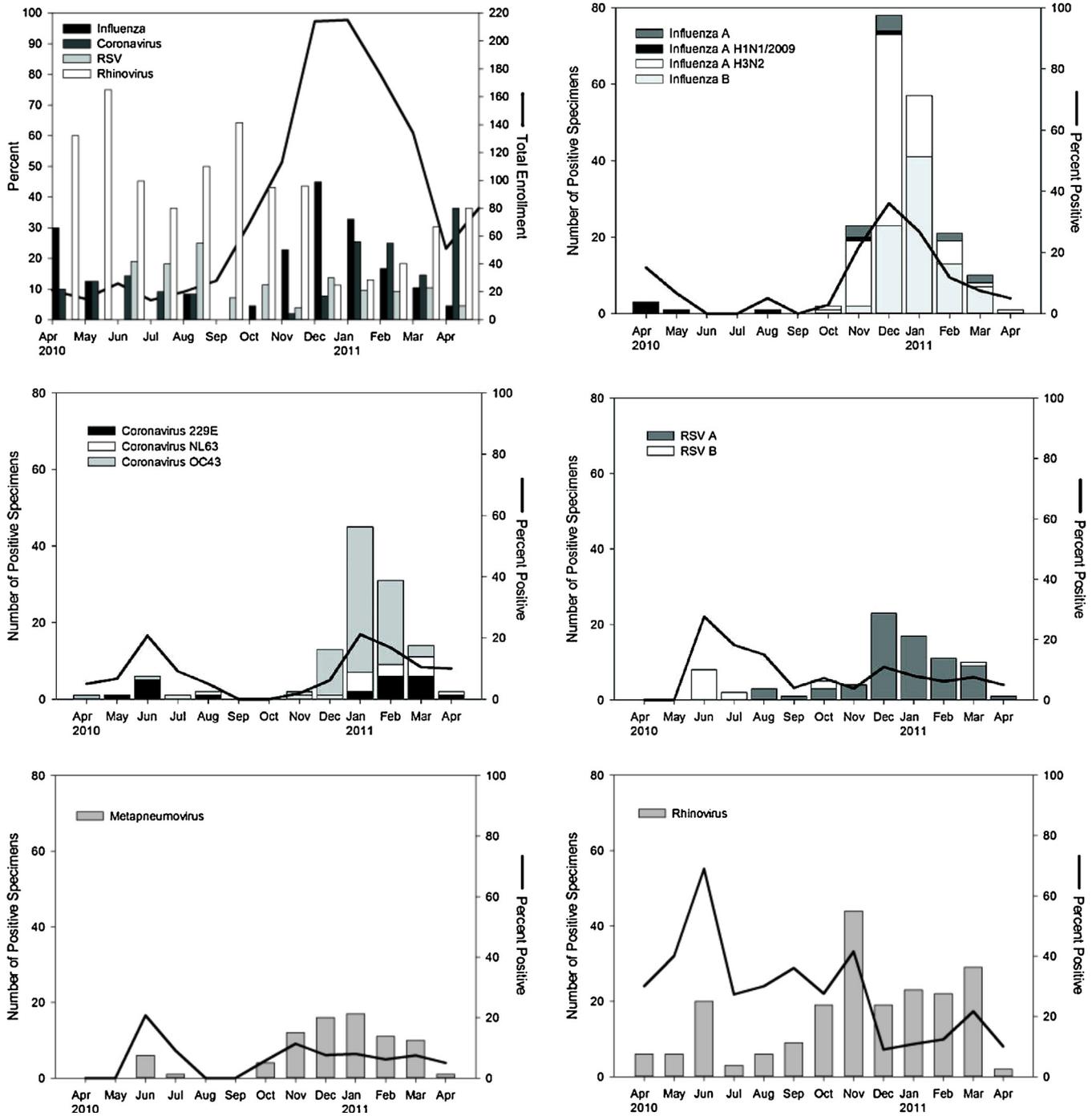


Figure 2. Monthly distribution of viral isolates. Total monthly enrollment (line), and the four most prevalent viruses (A). Monthly isolates by virus subtype (bars) and percent positive are demonstrated for the five most common viruses: influenza (B), coronavirus (C), respiratory syncytial virus (D), metapneumovirus (E), and rhinovirus (F).

influenza.³³ Twenty-two percent of all subjects with isolated coronavirus were hospitalized, but in the population aged ≥ 60 years this increased to 73%. While our study design precludes the determination of exact hospitalization rates as a result of coronavirus infections, the hospitalization rates were similar to those for influenza.

No pathogen was identified in 35% of subjects. This was more common in adults (no pathogen detected in 41% (321/793) of all adult subjects aged ≥ 18 years) than in children (no pathogen detected in 20% of subjects). It is unclear if these subjects had bacterial infections, pathogens not tested for in our assay, or non-infectious etiologies of their ILI. Unfortunately the lack of paired

sputum limited our ability to determine etiology. In a cohort in New Caledonia, paired sputum samples were required to determine the etiologic agent in 23% of subjects with lower respiratory tract infections.³² In a population of hospitalized children in Mexico with lower respiratory tract infections, viral etiologies were determined in only 47%.³⁴ This study found a virologic agent in 80% of children. This improved detection is likely in part due to the extended panel of viruses that were tested with the multiplex PCR.

More than one virus was isolated in 11.9% of subjects. It is not possible to determine the contribution of each virus to the clinical syndrome, nor is it possible to determine if some viruses that were

Table 3
Demographics and symptoms by age and pathogen

	Children				Adults			
	Coronavirus	Influenza	Rhinovirus	RSV	Coronavirus	Influenza	Rhinovirus	RSV
Number enrolled	12	43	29	34	65	107	132	23
Any antiviral	0%	9%	7%	6%	28%	33%	27%	35%
Chronic medical condition	42%	28%	38%	26%	42%	44%	63%	74%
Asthma	8%	7%	10%	3%	11%	21%	19%	26%
CVD	0%	0%	10%	6%	15%	10%	20%	17%
COPD	0%	0%	0%	0%	5%	6%	2%	13%
DM	0%	0%	0%	0%	8%	6%	19%	13%
Antibiotics	50%	44%	52%	71%	40%	48%	66%	65%
Inhaled steroids	8%	9%	21%	24%	18%	20%	14%	35%
Systemic steroids	25%	9%	17%	21%	22%	24%	28%	43%
Pregnant	0%	0%	0%	0%	0%	1%	0%	0%
Non-smoker	0%	0%	0%	0%	72%	51%	60%	65%
Current/former smoker	0%	0%	0%	0%	28%	49%	40%	35%
Seasonal flu vaccine	50%	47%	45%	38%	60%	39%	39%	52%
Outpatient	67%	81%	38%	35%	68%	67%	52%	57%
Hospitalized	33%	19%	62%	65%	32%	33%	48%	43%
Symptoms								
Rales/crepitations	33%	16%	55%	79%	25%	29%	39%	26%
Wheezing	25%	16%	21%	29%	12%	22%	25%	35%
Fever	67%	95%	69%	100%	45%	79%	65%	70%
Productive cough	75%	65%	83%	88%	52%	59%	58%	57%
Diarrhea	0%	9%	0%	12%	6%	11%	4%	4%
Dyspnea	0%	16%	31%	38%	22%	33%	39%	43%
Fatigue	0%	58%	34%	32%	71%	64%	41%	57%
Headache	0%	51%	21%	12%	74%	75%	55%	39%
Nausea	17%	42%	14%	24%	15%	21%	9%	22%
Red eyes	0%	49%	17%	9%	37%	27%	13%	17%
Sore throat	25%	60%	14%	15%	65%	61%	47%	52%

COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DM, diabetes mellitus; RSV, respiratory syncytial virus.

detected were not causing disease. While PCR significantly improves the sensitivity of detecting respiratory viruses, the detection of viral nucleic acids may not always represent causation. In prior case–control studies, virus nucleic acids could be isolated from 20–27% of asymptomatic subjects.^{35,36} This might be supported by the data showing that among those with mixed infections, the outcomes were not significantly worse than those

for subjects with single infections. While all subjects enrolled were symptomatic, and this would increase the likelihood that isolated pathogens were causative, cohort studies such as ours are not able to directly determine causation. With 62 unique combinations in 125 different subjects, the assessment of outcomes lacks statistical power to determine differences between the various combinations of mixed infections.

Table 4
Hospitalizations and deaths by pathogen

Pathogen	Hospitalized % (n)			Death % (n)		
	Age <18	Age 18–59	Age ≥60	Age <18	Age 18–59	Age ≥60
Adenovirus	41.8% (7)	66.7% (10)	100% (3)		6.7% (1)	
<i>Bordetella pertussis</i>	100% (2)	33.3% (1)				
Coronavirus	33.3% (4)	24% (12)	73.3% (11)		2% (1)	13.3% (2)
229E		45.5% (5)	100% (2)			
NL63		25% (2)	100% (1)			
OC43	40% (4)	16.1% (5)	66.7% (8)		3.2% (1)	16.7% (2)
Influenza	25.6% (11)	43.8% (39)	72.2% (13)			11.1% (2)
A		60% (3)				
A H1N1 2009		80% (4)				
A H3N2	25% (4)	50% (23)	72.7% (8)			9.1% (1)
B	29.2% (7)	27.3% (9)	83.3% (5)			16.7% (1)
Metapneumovirus	63.6% (7)	50% (11)	88.9% (8)			22.2% (2)
<i>Mycoplasma pneumoniae</i>	100% (4)	100% (1)	100% (1)			
Parainfluenza virus	66.7% (4)	41.7% (5)	100% (2)	16.7% (1)		
1						
2						
3	66.7% (4)	42.9% (3)	100% (1)	16.7% (1)		
4		40% (2)	100% (1)			
Rhinovirus	58.6% (17)	59% (59)	81.3% (26)		7% (7)	12.5% (4)
Respiratory syncytial virus	76.5% (26)	53.3% (8)	100% (8)	5.9% (2)		25% (2)
A	75.8% (25)	53.3% (8)	100% (6)	6.1% (2)		33.3% (2)
B	100% (1)		100% (2)			
Mixed infections	49% (25)	56.7% (34)	100% (14)		10% (6)	7.1% (1)
Negative cases	34.6% (18)	51.7% (123)	88% (73)		4.6% (11)	27.7% (23)
Total	46.2% (121)	49.8% (306)	84.1% (159)	1.5% (4)	4.2% (26)	19% (36)

Table 5
Outcomes

Variable at baseline	Patients who survived Median (range), n=999	Patients who died Median (range), n=66	Odds ratio Estimate (95% CI)	p-Value
Creatinine phosphokinase (U/l)	95 (9–7160)	90.5 (10–1840)	1.04 (1.00, 1.08)	0.066
Creatinine (mg/dl)	0.6 (0–14)	1.1 (0.1–9.7)	1.37 (1.19, 1.58)	<0.001
C-reactive protein (mg/l)	1.4 (0–36)	14.25 (1.6–34.1)	1.12 (1.09, 1.16)	<0.001
Hematocrit (%)	41.7 (14.3–76.1)	34.9 (13.2–57.5)	0.73 (0.66, 0.8)	<0.001
Hemoglobin (g/dl)	14.1 (4.1–25.4)	11.7 (4.7–19)	0.73 (0.66, 0.8)	<0.001
Lactate dehydrogenase (IU/l)	224 (26–4003)	338 (31–5175)	1.19 (1.08, 1.32)	0.001
Lymph (%)	18 (0–92)	7 (1–35)	0.92 (0.90, 0.95)	<0.001
Lymph absolute ($\times 10^9/l$)	1.4 (0–35.1)	0.7 (0.02–6.8)	0.92 (0.88, 0.95)	<0.001
Neutrophil (%)	70 (0–97)	87 (16–98)	1.06 (1.04, 1.08)	<0.001
Neutrophil absolute ($\times 10^9/l$)	5.7 (0–26.9)	8.6 (0.27–27.2)	1.01 (1.00, 1.01)	0.001 ^a
Platelets ($\times 10^9/l$)	231 (10–744)	140 (3–551)	0.91 (0.88, 0.94)	<0.001
White blood count ($\times 10^9/l$)	8.5 (0.1–54)	10.4 (0.3–29.2)	1.03 (0.99, 1.07)	0.179
	Patients who survived Percent	Patients who died Percent	Odds ratio Estimate (95% CI)	p-Value
Any antiviral medications	21.2	39.4	2.41 (1.44, 4.05)	<0.001 ^a
Inhaled steroids	18.4	18.2	1.00 (0.52, 1.91)	1.00
Systemic steroids	23.7	36.4	1.88 (1.11, 3.1)	0.02
History of smoking	30.3	50.0	2.30 (1.39, 3.79)	<0.001 ^a
Medical history				
Asthma	15.0	1.5	0.09 (0.01, 0.63)	0.02 ^a
Cardiovascular disorder	12.7	30.3	2.99 (1.71, 5.21)	<0.001 ^a
COPD	3.0	9.1	3.23 (1.29, 8.06)	0.01 ^a
Diabetes	8.7	18.2	2.33 (1.20, 4.52)	0.01
Immunodeficiency	3.7	16.7	5.20 (2.52, 10.75)	<0.001
Renal disorder	3.5	10.6	3.27 (1.39, 7.67)	0.01 ^a
Signs and symptoms				
Productive cough	62.9	51.5	0.63 (0.38, 1.03)	0.07
Diarrhea	7.5	3.0	0.39 (0.09, 1.60)	0.19
Dyspnea	32.5	39.4	1.35 (0.81, 2.25)	0.25
Fatigue	51.8	42.4	0.69 (0.42, 1.14)	0.14
Fever	70.9	75.8	1.28 (0.72, 2.29)	0.40
Headache	53.2	21.2	0.24 (0.13, 0.43)	<0.001
Nausea	18.9	1.5	0.07 (0.01, 0.48)	0.01 ^a
Rales/crepitations	36.7	83.3	8.61 (4.45, 16.66)	<0.001
Red eyes	21.6	4.5	0.17 (0.05, 0.56)	<0.001 ^a
Sore throat	46.4	21.2	0.31 (0.17, 0.57)	<0.001
Wheezing	23.2	9.1	0.33 (0.14, 0.78)	0.01 ^a

CI, confidence interval; COPD, chronic obstructive pulmonary disease.

^a When analysis for deaths attributed to ILI, the *p*-value was no longer <0.05.

The determination of non-influenza respiratory viruses in ILI has several implications. First, increasing knowledge that acute respiratory illness and its associated morbidity are often caused by respiratory viruses may stimulate the development of more affordable rapid diagnostic tests as well as therapeutics for these viruses. With the consideration of increasing antibiotic resistance, the diagnosis of respiratory viruses could decrease the quantities of empiric broad-spectrum antibiotics used.

The aim of this study was not the epidemiological surveillance of respiratory diseases. We included only patients who by the severity of their illnesses sought medical attention. The attending physicians determined the need for hospitalization or outpatient treatment. This limited our population to a particular group of patients with clinical manifestations of the disease. With this strategy we seek to find the more serious cases. From this we believe we can more easily determine the factors associated with severe disease. It is a limitation of this study, however, that we lose a part of the population with mild or asymptomatic disease.

In conclusion, viruses that were traditionally believed to produce minor disease have the capacity, in certain subjects, to cause severe cases, as suggested by the high proportion of adult patients with rhinovirus infection who required in-hospital treatment. We believe that these findings mark the need for specific studies that will help us to better understand the behavior of these viruses, and the factors associated with a more serious presentation.

Acknowledgements

La Red is funded by the Mexico Ministry of Health and the U.S. National Institute of Allergy and Infectious Diseases. This project has been funded in part by funding provided by: CONACYT through FONSEC SSA/IMSS/ISSSTE No. 71260 and No. 127088. Intramural Research Programs of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) through a contract with Westat, Inc., Contract Number: HHSN2722009000031, Task Order Number: HHSN27200002. And through the National Cancer Institute, National Institutes of Health, 318 under Contract No. HHSN261200800001E.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, or Westat, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Ethical approval: The Ethics committee of each institution reviewed the study, and the study was conducted following the principles of the ICH GCP, Declaration of Helsinki, and the Mexican General Health Law. All subjects provided informed consent. All data was de-identified during data collection. The project was registered on clinicaltrials.gov (NCT01418287).

Conflict of interest: The authors have no competing interests to declare.

References

- Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quinones-Falconi F, Bautista E, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med* 2009;**361**:680–9.
- Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**360**:2605–15.
- World Health Organization. World health report 2002. Geneva: WHO; 2002.
- Monto AS. Epidemiology of viral respiratory infections. *Am J Med* 2002;**112**(Suppl 6A):4S–12S.
- Kammerer PE, Montiel S, Kriner P, Bojorquez I, Ramirez VB, Vazquez-Erlbeck M, et al. Influenza-like illness surveillance on the California–Mexico border, 2004–2009. *Influenza Other Respi Viruses* 2012;**6**:358–66.
- Ahmed JA, Katz MA, Auko E, Njenga MK, Weinberg M, Kapella BK, et al. Epidemiology of respiratory viral infections in two long-term refugee camps in Kenya, 2007–2010. *BMC Infect Dis* 2012;**12**:7.
- Watanabe AS, Carraro E, Candéias JM, Donalísio MR, Leal E, Granato CF, et al. Viral etiology among the elderly presenting acute respiratory infection during the influenza season. *Rev Soc Bras Med Trop* 2011;**44**:18–21.
- Juven T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 2000;**19**:293–8.
- Laguna-Torres VA, Gomez J, Ocana V, Aguilar P, Saldarriaga T, Chavez E, et al. Influenza-like illness sentinel surveillance in Peru. *PLoS One* 2009;**4**:e6118.
- Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults. *J Med Virol* 2008;**80**:1824–7.
- Thiberville SD, Ninove L, Vu Hai V, Botelho-Nevers E, Gazin C, Thirion L, et al. The viral etiology of an influenza-like illness during the 2009 pandemic. *J Med Virol* 2012;**84**:1071–9.
- Ren L, Gonzalez R, Wang Z, Xiang Z, Wang Y, Zhou H, et al. Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007. *Clin Microbiol Infect* 2009;**15**:1146–53.
- Razanajatovo NH, Richard V, Hoffmann J, Reynes JM, Razafitrimo GM, Randra-manana RV, et al. Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS One* 2011;**6**:e17579.
- Boivin G, Hardy I, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of a clinical case definition. *Clin Infect Dis* 2000;**31**:1166–9.
- Peltola V, Reunanen T, Ziegler T, Silvennoinen H, Heikkinen T. Accuracy of clinical diagnosis of influenza in outpatient children. *Clin Infect Dis* 2005;**41**:1198–200.
- Nakanishi M, Yoshida Y, Takeda N, Hirana H, Horita T, Shimizu K, et al. Community-acquired pneumonia distinguished from influenza infection based on clinical signs and symptoms during a novel (swine) influenza A/H1N1 pandemic. *Prim Care Respir J* 2011;**20**:421–6.
- Lee VJ, Yap J, Cook AR, Tan CH, Loh JP, Koh WH, et al. A clinical diagnostic model for predicting influenza among young adult military personnel with febrile respiratory illness in Singapore. *PLoS One* 2011;**6**:e17468.
- Shlomai A, Nutman A, Kotlovsky T, Schechner V, Carmeli Y, Guzman-Gur H. Predictors of pandemic (H1N1) 2009 virus positivity and adverse outcomes among hospitalized patients with a compatible syndrome. *Isr Med Assoc J* 2010;**12**:622–7.
- Dominguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, de la Torre A, et al. Critically ill patients with 2009 influenza A(H1N1) in Mexico. *JAMA* 2009;**302**:1880–7.
- Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 2009;**302**:1872–9.
- Webb SA, Pettit V, Seppelt I, Bellomo R, Bailey M, Cooper DJ, et al. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. *N Engl J Med* 2009;**361**:1925–34.
- Widmer K, Zhu Y, Williams JV, Griffin MR, Edwards KM, Talbot HK. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012;**206**:56–62.
- World Health Organization. CDC protocol of realtime RTPCR for swine influenza A (H1N1). 28 April 2009; revision 1 (30 April 2009). Atlanta, GA: WHO/CDC. Available at: http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf [accessed 29.06.09].
- Reijans M, Dingemans G, Klaassen CH, Meis JF, Keijndener J, Mulders B, et al. RespiFinder: a new multiparameter test to differentially identify fifteen respiratory viruses. *J Clin Microbiol* 2008;**46**:1232–40.
- Loens K, van Loon AM, Coenjaerts F, van Aarle Y, Goossens H, Wallace P, et al. Performance of different mono- and multiplex nucleic acid amplification tests on a multipathogen external quality assessment panel. *J Clin Microbiol* 2012;**50**:977–87.
- Lam WY, Yeung AC, Tang JW, Ip M, Chan EW, Hui M, et al. Rapid multiplex nested PCR for detection of respiratory viruses. *J Clin Microbiol* 2007;**45**:3631–40.
- Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. *BMJ* 1997;**315**:1060–4.
- Boivin G, Osterhaus AD, Gaudreau A, Jackson HC, Groen J, Ward P. Role of picornaviruses in flu-like illnesses of adults enrolled in an oseltamivir treatment study who had no evidence of influenza virus infection. *J Clin Microbiol* 2002;**40**:330–4.
- Carballal G, Videla CM, Espinosa MA, Savy V, Uez O, Sequeira MD, et al. Multicenter study of viral acute lower respiratory infections in children from four cities of Argentina, 1993–1994. *J Med Virol* 2001;**64**:167–74.
- Berkley JA, Munywoki P, Ngama M, Kazungu S, Abwao J, Bett A, et al. Viral etiology of severe pneumonia among Kenyan infants and children. *JAMA* 2010;**303**:2051–7.
- Mathisen M, Basnet S, Sharma A, Shrestha PS, Sharma BN, Valentiner-Branth P, et al. RNA viruses in young Nepalese children hospitalized with severe pneumonia. *Pediatr Infect Dis J* 2011;**30**:1032–6.
- Mermond S, Zurawski V, D'Ortenzio E, Driscoll AJ, DeLuca AN, Deloria-Knoll M, et al. Lower respiratory infections among hospitalized children in New Caledonia: a pilot study for the Pneumonia Etiology Research for Child Health project. *Clin Infect Dis* 2012;**54**(Suppl 2):S180–9.
- Cabeca TK, Bellei N. Human coronavirus NL-63 infection in a Brazilian patient suspected of H1N1 2009 influenza infection: description of a fatal case. *J Clin Virol* 2012;**53**:82–4.
- Noyola DE, Rodriguez-Moreno G, Sanchez-Alvarado J, Martinez-Wagner R, Ochoa-Zavala JR. Viral etiology of lower respiratory tract infections in hospitalized children in Mexico. *Pediatr Infect Dis J* 2004;**23**:118–23.
- Jansen RR, Wieringa J, Koekkoek SM, Visser CE, Pajkrt D, Molenkamp R, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol* 2011;**49**:2631–6.
- Sanghavi SK, Bullotta A, Husain S, Rinaldo CR. Clinical evaluation of multiplex real-time PCR panels for rapid detection of respiratory viral infections. *J Med Virol* 2012;**84**:162–9.