Journal of Clinical Virology 102 (2018) 27–31

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Epidemiology and seasonality of acute respiratory infections in hospitalized children over four consecutive years (2012–2016)

Adrien Fillatre[a], Catherine François[a,b], Christine Segard[a], Gilles Duverlie[a,b], Denise Hecquet[a], Christine Pannier[a], Catherine Roussel[a], Patricia Zawadzki[a], Etienne Brochot[a,b], Sandrine Castelain[a,b,*]

[a] Virology Department Centre de Biologie Humaine, Centre Hospitalo-Universitaire Amiens Picardie, Amiens, France
[b] EA4294, Université de Picardie Jules Verne, Amiens, France

ARTICLE INFO

Keywords:
Respiratory viruses
Coinfection
Epidemiology
Children
Seasons

ABSTRACT

Background: Acute respiratory infections are a principal cause of illness and mortality especially in young children worldwide.
Objectives: To study the epidemiology and seasonality of viral respiratory infections in hospitalized children (under the age of 16) between September 2012 and August 2016.
Study design: Nasopharyngeal swabs or aspirates were collected from 3199 symptomatic patients and then screened with a routine multiplex PCR assay.
Results: Respiratory viruses were detected for 1624 (50.8%) of the 3199 children in the study population. Of these, 210 (13.3%) were positive for two viruses, 28 (1.7%) were positive for three, and 3 (0.2%) were positive for four. The viral profile varied with age. Some viruses were significantly more frequent in children under the age of 1 month (such as human respiratory syncytial virus ($p < 0.0001$)), whereas others were significantly more frequent in children over that age (such as influenza viruses ($p < 0.0001$) and adenoviruses ($p = .0006$)). The distribution of viruses is variable over the year depending on the species. However, the atmospheric temperature was rarely found to be a limiting factor in the circulation of respiratory viruses.
Conclusions: our results constitute a detailed description of the distribution of respiratory viruses among hospitalized children over four consecutive years. Our data notably highlight the persistence of non-enveloped viruses and some enveloped viruses throughout the year–regardless of temperature variations.

1. Background

Over recent years, the development of multiplex molecular biology techniques has enabled the rapid detection of respiratory viruses in many different contexts. Indeed, the rapid identification of respiratory pathogens has modified and improved the preventive and curative management of patients. It has been clearly shown that the rapid, accurate identification of respiratory specimens can have a significant impact on disease progression; this enables the introduction of appropriate therapy, decreases the unnecessary use of antimicrobial agents, and thus limits the risk of secondary infections in medical establishments [1]. These multiplex techniques have supplanted traditional methods for the diagnosis of respiratory tract infections (such as cultures, direct fluorescent-antibody staining, and enzyme immunoassays) and are superior in terms of sensitivity, execution time and turn-around time [2]. Lastly, multiplex molecular biology techniques can also be rapidly adapted to detect new or emerging viruses. Although the reagent cost is inherently higher, many studies have clearly demonstrated that appropriately prescribed respiratory multiplex assays are cost-effective and can generate significant savings for hospitals [3–5].

The diagnostic spectrum varies from one commercial kit to another, although all kits detect the main pathogens: influenzavirus A (InfA), influenzavirus B (InfB), human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), enterovirus (EV), human rhinovirus (hRV), parainfluenzaviruses (PIVs), adenoviruses (AdVs), human bocavirus (hBoV), and human coronaviruses (229E, NL63, OC43, HKU1; hCoVs).

For all these reasons, the use of multiplex molecular assays has increased the detection of viral respiratory infections and co-infections. Although earlier estimates of viral coinfection (based on culture, serology and certain molecular methods) yielded a frequency of around 10% [6], values of up to 44% in young children have recently been

* Corresponding author at: Virologie, Centre de Biologie Humaine, Centre Hospitalo–Universitaire Amiens Picardie, F – 80054 Amiens cedex, France.
E-mail address: sandrine.castelain@u-picardie.fr (S. Castelain).

https://doi.org/10.1016/j.jcv.2018.02.010
Received 22 September 2017; Received in revised form 4 January 2018; Accepted 19 February 2018

Table 1
Proportion of positive samples in each age group for each virus, in samples from 2012 to 2016.

Patient age group (N = total number)	Number of samples		Percentage of positive samples								
	Negative	Positive for at least one virus (%)	Inf A	Inf B	PIV	hRSV	ER&hRV	AdV	hBoV	hCoV	hMPV
< 1 month (N = 602)	387	215 (35.7%)	3.77%	1.67%	9.21%	21.34%	52.72%	1.67%	1.26%	5.02%	3.35%
1–24 months (N = 1477)	651	826 (55.9%)	2.71%	2.21%	11.46%	13.87%	46.03%	4.62%	6.73%	7.44%	4.92%
> 24 months (N = 1120)	537	583 (52.0%)	5.51%	7.04%	8.58%	7.35%	47.01%	7.66%	7.66%	4.59%	4.59%

determined with PCR assays [7,8].

The primary objective of the present study was to assess the epidemiology and seasonality of viral respiratory infections in hospitalized children. In this context, we analysed the results of a routine, multiplex PCR assay (xTAG Respiratory Viral Panel Fast assay, Luminex Molecular Diagnostics) for respiratory viruses gathered over four consecutive years (from September 2012 to August 2016), and thus investigated the seasonal and year-to-year prevalences of the viruses most commonly responsible for respiratory infections and coinfections.

2. Study design

2.1. Patients and specimens

We performed a single-centre, retrospective study of children (under the age of 16) admitted to Amiens University Hospital (Amiens, France) for an acute respiratory illness. In accordance with local guidelines, a multiplex PCR assay for respiratory viruses was used to screen nasopharyngeal swabs or aspirates at time of respiratory symptoms. The samples were collected from 3199 patients between September 2012 and August 2016. All specimens were stored at −70 °C after testing.

2.2. Respiratory virus testing

Nasopharyngeal swabs or aspirates were extracted using the NucliSENS® easyMAG® technique (bioMérieux, Craponne, France). Before extraction, an internal positive control (*Escherichia coli* phage MS2, provided by the manufacturer) was added to the samples. The extracts were screened with the xTAG Respiratory Viral Panel Fast assay (Luminex Molecular Diagnostics Inc., Toronto, Canada), according to the manufacturer's instructions. An aliquot of each extract was stored at −70 °C. A positive run control (bacteriophage lambda DNA) was used in each run to monitor assay performance. The resulting median fluorescence intensities (MFIs) were analysed the Luminex 100 IS system and TDAS RVP Fast software (version 2.00, Luminex). The assay can detect 18 different respiratory viruses: InfA H1N1 and H3N2, InfB, hRSV, hMPV, EV/hRV, PIV types 1, 2, 3 and 4, AdV, hBoV, and hCoVs 229E, NL63, OC43 and HKU1.

2.3. Weather variables

Retrospective temperature data were obtained from Météo France, the French state meteorological office (http://www.meteofrance.com/climat/france/abbeville/80001001/releves).

2.4. Statistical analysis

A non-parametric Mann–Whitney test was applied to continuous variables, and a chi-squared test or Fisher's exact test was applied to categorical variables. The threshold for statistical significance (two-tailed) was set to $p \leq 0.05$ was considered.

2.5. Ethics statement

We performed out a non-interventional study; no additional diagnostic, monitoring or treatment procedures were applied to routine patient care. Data were analysed after they had been anonymized. In line with the French legislation on clinical studies, this type of trial does not require approval by an independent ethics committee or the provision of informed consent by the participants.

3. Results

3.1. The distribution of respiratory viruses as a function of the patient's age

In total, 3199 samples (received from September 2012 to August 2016) were analysed. Of these, 1575 (49.2%) were negative for all 18 respiratory viruses, whereas 1624 (50.8%) were positive for at least one respiratory virus. The total numbers of positive samples were as follows: 72 for InfA, 72 for InfB, 192 for PIV, 237 for hRSV, 891 for ER/hRV, 100 for AdV, 120 for hBoV, 116 for hCoV, and 87 for hMPV.

As shown in Table 1, the viral profile varied with age. Some viruses were significantly more frequent in children under the age of 1 month (such as hRSV ($p < 0.0001$)), whereas others were significantly more frequent in children over that age (such as InfA/InfB ($p < 0.0001$) and AdVs ($p = .0006$)). The respective frequencies of other viruses (such as the hCoV, hMPV, PIVs, and ER/hRV) did not vary with the patient's age.

Multiplex molecular assays are valuable because they enable the simultaneous detection of several different viruses in the same sample, and can therefore reveal the presence of coinfections. Here, 210 samples (13.3%) were positive for two different viruses, 28 (1.7%) were positive for three, and 3 (0.2%) were positive for four. The percentage of patients with a coinfection differed significantly as a function of age ($p < 0.0001$): 9.5% for children younger than 1 month, 63.7% for children aged between 1 and 24 months, and 26.8% for children aged over 24 months. The viral profile in coinfections (Fig. 1) highlighted the predominance of EV/hRV. Indeed, EV/hRV was found in 161 (76.6%) of the 210 coinfections with two viruses, in 25 of the 28 (89.3%) coinfections with three viruses, and 3 of the 3 (100%) infections with four viruses. The viruses most frequently detected with EV/hRV were the PIV types 1–4 (17.1%), followed by hRSV (15.7%), AdV (14.3%), hBoV (11.4%), hCoVs (7.1%), and hMPV (7.6%).

In the absence of EV/hRV, the most frequently observed association was hRSV and hCoVs (3.3%). Conversely, some associations were never observed (e.g. InfB with hRSV, PIV or hCoVs, and InfA with hMPV).

3.2. Changes in the viral distribution profile over time

This retrospective study was performed over 4 consecutive years (September 2012 to August 2016), making it possible to study the seasonal and year-to-year distribution of various viruses in a region with an oceanic, temperate climate.

Unsurprisingly for the northern hemisphere, the number of assays performed was higher in winter than in summer (Table 2). The percentage of positive assays was highest in autumn (mid September to mid December) (55%), followed by winter (mid December to mid March) (53.6%), spring (mid March to mid June) (50.5%) and summer (mid June to mid December) (39.5%). The number of viral co-infections was highest in winter (18.3%), followed by spring (15.8%), autumn (11.7%) and summer (11.6%).