

Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses

Susanna Esposito,^a Cristina Daleno,^a Giulia Prunotto,^a Alessia Scala,^a Claudia Tagliabue,^a Irene Borzani,^b Emilio Fossali,^a Claudio Pelucchi,^c Nicola Principi^a

^aDepartment of Maternal and Pediatric Sciences, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. ^bPediatric Radiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. ^cDepartment of Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy.

Correspondence: Nicola Principi, Department of Maternal and Pediatric Sciences, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Commenda 9, 20122 Milano, Italy. E-mail: nicola.principi@unimi.it

Accepted for publication 19 December 2011. Published Online 13 February 2012.

Background Little is known about the prevalence of viral infections in children with community-acquired pneumonia (CAP).

Objectives To describe the clinical and virological data collected from children with radiographically confirmed CAP in whom 17 respiratory viruses were sought in respiratory secretion samples during the acute phase of the disease.

Patients and methods The study involved 592 children with radiographically confirmed CAP whose respiratory secretion samples were tested using the Luminex xTAG Respiratory Virus Panel Fast assay, which simultaneously detects influenza A virus, influenza B virus, respiratory syncytial virus (RSV)-A and -B, parainfluenzavirus-1, -2, -3, and -4, adenovirus, human metapneumovirus, coronaviruses 229E, NL63, OC43, and HKU1, enterovirus/rhinovirus, and bocavirus. A real-time PCR assay was used to identify the rhinovirus in the enterovirus/rhinovirus-positive samples.

Results A total of 435 children (73.5%) were positive for at least one virus: the most frequently detected was RSV, which was found in 188 (31.7%), followed by rhinovirus ($n = 144$, 24.3%), bocavirus ($n = 60$, 10.1%), influenza viruses ($n = 57$, 9.6), and hMPV ($n = 49$, 8.2%). Viral co-infections were found in 117 children (19.7% of the enrolled children; 26.9% of those with viral infections). Marginal differences were found between the infections owing to a single virus. Co-infections showed radiographic evidence of alveolar pneumonia significantly more frequently than single infections (OR 1.72, 95% CI 1.05–2.81).

Conclusions The findings of this study highlight the importance of respiratory viruses (mainly RSV and rhinovirus) in children with CAP and show the characteristics of both the single infections and co-infections associated with the disease.

Keywords Children, community-acquired pneumonia, pediatrics, respiratory infections, respiratory viruses, viral infections.

Please cite this paper as: Esposito *et al.* (2013) Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses. *Influenza and Other Respiratory Viruses* 7(1), 18–26.

Background

Despite the development of effective antimicrobial therapy, management guidelines, and effective vaccines, community-acquired pneumonia (CAP) remains one of the major causes of morbidity in children living in developed countries, and one of the main causes of mortality in those living in the developing world.^{1–3} Detailed information concerning the etiology of CAP is required in order to formulate treatment recommendations and implement preventive measures. Evaluating the relative importance of each potential pathogen may also contribute to improving our understanding of the pathogenesis of the disease.

Viruses have long been considered the predominant pathogens of CAP, particularly in infants and children aged <5 years.^{4,5} However, because of the limited sensitivity and specificity of previous virological diagnostic techniques based on cultures or immunofluorescence microscopy and the measurement of antibody responses in paired serum samples, a considerable number of CAP cases were not etiologically identified. Recent advances in molecular diagnosis have made it possible to detect previously unknown viral agents and define the epidemiology of the most common respiratory viral infections more precisely,⁶ but there are still many unanswered questions concerning the role of viruses in pediatric CAP. Little is known about the real

prevalence of some viral infections, particularly those that have been identified more recently, and the frequency and importance of viral co-infections have not been fully clarified. In order to answer these questions, there is a need for studies using methods that allow the identification of the greatest number of potential viral respiratory pathogens.

The aim of this study was to describe the data collected from children with radiographically confirmed CAP in whom 17 respiratory viruses were detected in respiratory secretion samples using the Luminex xTAG Respiratory Virus Panel (RVP) Fast assay during the acute phase of the disease.

Patients and methods

Patient enrollment

The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and was carried out in the Department of Maternal and Pediatric Sciences of the University of Milan between November 1 and April 30 of four consecutive winter and early spring seasons (2007–2008, 2008–2009, 2009–2010, and 2010–2011). The written informed consent of a parent or legal guardian was required, and the older children were asked to give their assent.

All of the children aged between 1 month and 14 years seen in the emergency room (ER) of the Department of Maternal and Pediatric Sciences who had fever (an axillary temperature of $>38^{\circ}\text{C}$), and signs and symptoms consistent with CAP (i.e., cough, tachypnea, dyspnea or respiratory distress, and breathing with grunting or wheezing sounds with rales), were considered eligible for the study. The exclusion criteria were chronic diseases increasing the risk of respiratory infections, including premature birth; chronic disorders of the pulmonary or cardiovascular systems, including asthma; chronic metabolic diseases, including diabetes mellitus; neoplasia; kidney or liver dysfunction; hemoglobinopathies; immunosuppression; diseases requiring long-term aspirin therapy; and genetic or neurological disorders. The children with presumed nosocomial CAP (i.e., appearing more than 48 hours after admission or within 2 weeks of hospital discharge) were also excluded.

All of the chest X-rays were evaluated by an independent expert radiologist who classified the findings as alveolar pneumonia, non-alveolar pneumonia, or no pneumonia in accordance with the World Health Organization (WHO) criteria for a standardized interpretation of pediatric chest radiographs for the diagnosis of pneumonia.⁷ Alveolar pneumonia was defined as dense opacity appearing as the fluffy consolidation of a part or all of a lobe or entire lung, often containing air revealed by bronchography, and sometimes associated with pleural effusion.⁷

Respiratory secretion samples were taken from the enrolled children with radiographically confirmed CAP using a pernasal nylon flocked swab and stored in a tube of UTM-RT (Cat. No. 360c; Copan Italia, Brescia, Italy).^{8–12} Upon enrollment, detailed information regarding their demographic data, clinical history, and the clinical characteristics of the disease was collected together with a blood sample for the evaluation of laboratory variables, including white blood cell counts, C-reactive protein (CRP), and a blood culture. The drug treatment was chosen by the pediatrician in charge on the basis of the guidelines of the Italian Society of Pediatrics.¹³ Children with mild disease were sent home with detailed therapeutic instructions and a recommendation to return to the hospital in the case of persistent fever or worsening respiratory symptoms and signs. The children with severe disease or family problems were hospitalized.

The clinical data collected during hospitalization were recorded daily, and all of the children (whether hospitalized or sent home immediately after enrollment) were re-evaluated 15 ± 2 days later by means of interviews and clinical examinations were carried out by trained investigators using standardized questionnaires that also collected information regarding household illnesses and related morbidities.

Virus identification

Viral RNA or DNA was extracted from the respiratory secretions by means of a Nuclisens EasyMAG automated extraction system (Biomerieux, Craaponne, France) and was then tested using the Luminex xTAG Respiratory Virus Panel (RVP) Fast assay in accordance with the manufacturer's instructions (Luminex Molecular Diagnostics Inc., Toronto, ON, Canada). All of the xTAG RVP Fast reagents were provided by Abbott GmbH & Co. (Wiesbaden-Delkenheim, Germany). The RVP Fast assay consisted of a single multiplex polymerase chain reaction (PCR) with labeled primers, followed by the single-step hybridization of the PCR products with the fluorescent bead array and incubation with the reagents. The plate was then analyzed using a Bio-Plex 200 System (Bio-rad Laboratories, Milan, Italy) and its associated software Luminex xPONENT version 3.1 (Luminex Molecular Diagnostics Inc., provided by Abbott), and its median fluorescent intensity (MFI) was determined. An MFI above the threshold level determined by the manufacturer for a particular target indicated a positive result for that target. The mean fluorescence intensities establishing positivity were established using Tag-It data analysis software (TDAS, Luminex).

The RVP Fast assay simultaneously detects influenza A virus (subtypes H1 or H3), influenza B virus, respiratory syncytial virus (RSV)-A and -B, parainfluenzavirus-1, -2, -3, and -4, adenovirus, human metapneumovirus (hMPV),

coronaviruses 229E, NL63, OC43, and HKU1, enterovirus/rhinovirus, and human bocavirus.^{14,15} The assay also tests an internal positive control added to each specimen at the extraction stage (*Escherichia coli* phage MS2 RNA) and a positive run control added to each plate (bacteriophage lambda DNA).

The enterovirus/rhinovirus-positive samples were retested in order to identify the rhinovirus. This real-time PCR assay was performed using the iAg-Path-ID one-step RT-PCR Kit (Applied Biosystems, Foster City, CA, USA), and the primers and probe sequences were those reported by Lu *et al.*¹⁶ Briefly, each 25 μ l reaction mixture contained 1 μ m of forward and reverse primers, 0.1 μ m of the probe, and 5 μ l of nucleic acid extract. Amplification was performed using a 7900 HT Fast Real-Time PCR detection system (Applied Biosystems) and the following thermocycling conditions: 10 min at 48°C for RT, 3 min at 95°C for polymerase activation, and then 45 cycles of 15 seconds at 95°C and 1 minutes at 60°C. Each run included template and non-template controls.

Statistical analysis

Descriptive statistics were generated. The continuous variables are given as mean values \pm standard deviation (SD) and were analyzed using a two-sided, non-parametric Wilcoxon rank sum test or, when the data were normally distributed (on the basis of the Shapiro–Wilk statistic), a two-sided Student's *t*-test; the categorical variables are given as absolute numbers and percentages, and were compared between groups using contingency table analysis and the chi-square test or Fisher's exact test as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure the associations between positivity for the selected viruses and (i) the presence of fever, (ii) hospitalization, (iii) the presence of rales, (iv) the presence of wheezes, (v) having a relative with a similar illness, and (vi) radiological evidence of alveolar pneumonia. The ORs were obtained using unconditional multiple logistic regression analysis, adjusted for age (<1, 1–3, and \geq 4 years) and gender. We also analyzed the association between the presence of more than one viral infection and the same clinical and radiological characteristics, once again adjusting for age and gender. All of the analyses were made using SAS version 9.1 (Cary, NC, USA).

Results

During the 4 years of the study, of the 592 children (311 boys; mean age \pm SD 3.2 \pm 3.0 years) with radiographically confirmed CAP, 435 (73.5%) were positive for at least one virus. Viruses were detected in 120/132 children aged <1 year (90.9%), 235/293 aged 1–3 years (80.2%), and 89/167 aged \geq 4 years (47.9%; $P < 0.05$ versus the <1 year

and 1–3 year age groups). Nine children (all negative for respiratory viruses) had positive blood cultures (six for *Streptococcus pneumoniae*, two for *Staphylococcus aureus*, and one for non-typeable *Haemophilus influenzae*). As a preliminary analysis of the prevalence of respiratory viruses showed that there were no statistically significant differences by year of sample collection, all of the data collected in the 4 years were considered together.

Table 1 shows the viral findings by age group and the presence of viral–viral co-infections. The most frequently detected virus was RSV, which was found in 188 children (31.7%), followed by rhinovirus ($n = 144$, 24.3%), bocavirus ($n = 60$, 10.1%), influenza viruses ($n = 57$, 9.6%: six seasonal A/H1N1, 17 A/H3N2, eight B, and 26 pandemic A/H1N1), hMPV ($n = 49$, 8.2%), coronaviruses ($n = 33$, 5.6%: 20 OC43, five 229E, five NL63, and three HKU1), enterovirus ($n = 21$, 3.5%), adenovirus ($n = 11$, 1.8%), and parainfluenza viruses ($n = 11$, 1.8%: seven parainfluenza-4, one parainfluenza-1, one parainfluenza-2, and one parainfluenza-3). Among the children aged 4–14 years, rhinovirus was the most common cause of infection.

All of the studied viruses were found as isolated agents and in co-infections. The incidence of co-infections decreased with age and was significantly lower in the children aged 4–14 years (10/80, 12.5%) than in those aged <1 year (42/120, 35.0%; $P < 0.05$) or 1–3 years (65/235, 27.7%; $P < 0.05$). The virus causing the highest percentage of co-infections involved coronavirus, which was found together with other viruses in 26/33 children (78.8%), followed by bocavirus (45/60, 75%), parainfluenza viruses (6/11, 54.5%), adenovirus (6/11, 54.5%), enterovirus (11/21, 52.3%), rhinovirus (66/144, 45.8%), RSV (76/188, 40.4%), hMPV (11/49, 22.4%), and influenza viruses (9/57, 15.8%).

Table 2 summarizes the viral co-infections found in 117 children (19.7% of the enrolled children and 26.9% of those with viral infections): 96 were infected by two, 20 by three, and one by four viruses. The most common co-infections were those involving RSV and rhinovirus (32 cases, 27.3%), rhinovirus and bocavirus (11 cases, 9.4%), RSV and coronavirus (11 cases, 9.4%), and RSV and bocavirus (10 cases, 8.5%).

Table 3 describes the characteristics of the CAP associated with single viral infections. In order to be able to evaluate sufficiently substantial numbers, we only compared the single infections detected in at least 15 cases. Analysis of the differences between infections owing to RSV, rhinovirus, influenza, hMPV, and bocavirus showed that the mean age of the children with RSV-positive CAP was significantly lower than that of those with rhinovirus- or influenza-positive CAP ($P < 0.05$); high-grade fever ($\geq 39^\circ\text{C}$) was significantly more frequent among the children with hMPV-positive CAP than among those with rhinovirus-positive

Table 1. Viral findings in children admitted to emergency room because of community-acquired pneumonia (CAP)

Viral type	Age <1 year		Age 1–3 years		Age 4–14 years		Total	
	Total no. (%)	Co-infected no. (%)	Total no. (%)	Co-infected no. (%)	Total no. (%)	Co-infected no. (%)	Total no. (%)	Co-infected no. (%)
RSV	66 (11.1)	27 (40.9)	105 (17.7)	44 (41.9)	17 (2.9)	5 (29.4)	188 (31.7)	76 (40.4)
Rhinovirus	41 (6.9)	26 (63.4)*	69 (11.6)	33 (47.8)*	34 (5.8)	7 (20.6)	144 (24.3)	66 (45.8)
Bocavirus	12 (2.0)	11 (91.7)	45 (7.6)	31 (68.9)	3 (0.5)	3 (100.0)	60 (10.1)	45 (75.0)
Influenza viruses	13 (2.2)	4 (30.8)*	26 (4.4)	4 (15.4)*	18 (3.0)	1 (5.6)	57 (9.6)	9 (15.8)
A/H1N1s	1 (0.1)	0	4 (0.7)	2 (50.0)	1 (0.1)	1 (100.0)	6 (1.0)	3 (50.0)
A/H3N2	3 (0.5)	1 (33.3)	8 (1.3)	1 (12.5)	6	0	17 (2.9)	2 (11.8)
B	2 (0.3)	1 (50.0)	3 (0.5)	0	3 (0.5)	0	8 (1.3)	1 (12.5)
A/H1N1v	7 (1.2)	2 (28.6)	11 (1.8)	1 (9.1)	8 (1.3)	0	26 (4.4)	3 (11.5)
hMPV	15 (2.5)	4 (26.7)	25 (4.2)	6 (24.0)	9 (1.5)	1 (11.1)	49 (8.2)	11 (22.4)
Coronavirus	13 (2.2)	13 (100.0)*	14 (2.4)	11 (78.6)*	6 (1.0)	2 (33.3)	33 (5.6)	26 (78.8)
OC43	9 (1.5)	9 (100.0)	7 (1.2)	6 (85.7)	4 (0.7)	2 (50.0)	20 (3.4)	17 (85.0)
229E	1 (0.1)	1 (100.0)	3 (0.5)	2 (66.6)	1 (0.1)	0	5 (0.8)	3 (60.0)
NL63	3 (0.5)	3 (100.0)	2 (0.3)	1 (50.0)	0	0	5 (0.8)	4 (80.0)
HKU1	0	0	2 (0.3)	2 (100.0)	1 (0.1)	0	3 (0.5)	2 (66.6)
Adenovirus	3 (0.5)	3 (100.0)	7 (1.2)	2 (28.6)	1 (0.1)	1 (100.0)	11 (1.8)	6 (54.5)
Enterovirus	5 (0.8)	2 (40.0)	13 (2.2)	8 (61.5)	3 (0.5)	1 (33.3)	21 (3.5)	11 (52.4)
Parainfluenza	1 (0.1)	1 (100.0)	9 (1.5)	4 (44.4)	1 (0.1)	1 (100.0)	11 (1.8)	6 (54.5)
1	0	0	1 (0.1)	0	0	0	1 (0.1)	0
2	0	0	1 (0.1)	0	1 (0.1)	1 (100.0)	2 (0.3)	1 (50.0)
3	0	0	1 (0.1)	1 (100.0)	0	0	1 (0.1)	1 (100.0)
4	1 (0.1)	1 (100.0)	6 (1.0)	3 (50.0)	0	0	7 (1.2)	4 (57.1)

RSV, respiratory syncytial virus; hMPV, human metapneumovirus.

* $P < 0.05$ versus age group 4–14 years.

CAP ($P < 0.05$); and rales were significantly more frequent among the children with RSV-, hMPV-, and bocavirus-positive CAP than among those with rhinovirus-positive CAP ($P < 0.05$). Influenza-positive CAP cases showed similar diseases among family members significantly more often than the rhinovirus- and bocavirus-positive cases ($P < 0.05$). There were no other between-group differences in terms of clinical presentation or outcomes.

Among the laboratory parameters, CRP levels were significantly higher in the rhinovirus-positive cases than in the RSV- and influenza-positive cases ($P < 0.05$); there were no differences in white blood cell counts.

In relation to the radiographic findings, alveolar pneumonia was significantly more frequent in the rhinovirus-positive cases than in the influenza- and hMPV-positive cases ($P < 0.05$).

Multivariate analysis showed that the rhinovirus-positive cases were significantly less frequently associated with fever (OR 0.37, 95% CI 0.21–0.63), rales (OR 0.37, 95% CI 0.21–0.63), or a similar illness among family members (OR 0.37, 95% CI 0.19–0.72) than rhinovirus-negative cases. On the contrary, hMPV-positive cases were significantly more frequently associated with fever (OR 3.11, 95% CI 1.24–7.80) than hMPV-negative cases, and influenza-positive cases

were significantly more frequently associated with a similar illness among family members (OR 3.24, 95% CI 1.70–6.20) and significantly less frequently associated with alveolar pneumonia (OR 0.38, 95% CI 0.18–0.80) than influenza-negative cases.

Table 4 shows the demographic, clinical, laboratory, and radiographic variables associated with the single viral infections and co-infections. The only significant differences were mean age (which was significantly higher in the children with a single viral infection than in those with co-infections) ($P < 0.001$), and the radiographic finding of alveolar pneumonia, which was significantly more frequent in the children with co-infections ($P < 0.05$). Multivariate analysis showed that the only significant association was the significantly more frequent radiographic evidence of alveolar pneumonia in the children with co-infections than in those with single infections (OR 1.72, 95% CI 1.05–2.81).

Discussion

We used a combination of two molecular methods to identify 17 viruses in upper respiratory tract secretions in order to evaluate the relationships between viral infections and

Table 2. Viral co-infections

Associations	Prevalence, no. (%)
Dual infections	96/592 (16.2)
RSV + rhinovirus	32
Rhinovirus + bocavirus	11
RSV + coronavirus	11
RSV + bocavirus	10
hMPV + bocavirus	4
Rhinovirus + parainfluenza	4
RSV + influenza	3
Enterovirus + bocavirus	3
RSV + hMPV	2
RSV + enterovirus	2
Coronavirus + bocavirus	2
Rhinovirus + adenovirus	2
Rhinovirus + influenza	3
RSV + adenovirus	1
Rhinovirus + hMPV	1
Influenza + Enterovirus	1
Adenovirus + bocavirus	1
Adenovirus + coronavirus	1
Parainfluenza + bocavirus	1
Parainfluenza + hMPV	1
Triple infections	20/592 (3.4)
RSV + rhinovirus + coronavirus	4
RSV + rhinovirus + bocavirus	3
RSV + bocavirus + enterovirus	3
RSV + bocavirus + coronavirus	3
Rhinovirus + influenza + bocavirus	2
RSV + hMPV + enterovirus	1
Rhinovirus + bocavirus + coronavirus	1
Rhinovirus + adenovirus + coronavirus	1
Rhinovirus + hMPV + coronavirus	1
hMPV + coronavirus + enterovirus	1
Quadruple infection	1/592 (0.2)
RSV + rhinovirus + bocavirus + coronavirus	1

RSV, respiratory syncytial virus; hMPV, human metapneumovirus.

the development of CAP in a considerable number of otherwise healthy infants and children. The enrollment of a large number of pediatric patients was facilitated by the use of pernasal nylon flocked swabs, which are significantly less invasive than nasopharyngeal aspirates and washes, and a valid alternative means of collecting secretions for the detection of respiratory viruses, particularly when used with molecular assays.^{8–12} To the best of our knowledge, we evaluated more respiratory viruses (including recently identified viruses) in children with CAP than ever before, thus improving our understanding of the role of individual viruses and viral co-infections. The data not only confirm the importance of viruses in determining pediatric CAP previously highlighted by a number of authors, but also suggest that viruses may play an even greater role than pre-

viously thought as more than 75% of the children had a viral respiratory infection, including more than 90% of those aged <12 months. Previous studies have always found lower percentages and have therefore underestimated the real importance of viruses in pediatric CAP, probably because they sought a smaller number of viruses.^{17–25}

RSV and rhinovirus were the most common pathogens associated with CAP in the form of single viral infections and co-infections. They were found alone in more than 50% of the cases, thus highlighting their role as the causes of pediatric CAP and underlining the need to develop safe and effective vaccines in order to face the clinical problems they may give rise to. This seems to be particularly important in the case of rhinovirus which, until a few years ago, was considered only relatively important, was rarely associated with CAP, and was not usually included in the evaluation of CAP etiology.¹

The most frequently identified of the recently discovered viruses were bocavirus and hMPV. The data regarding bocavirus are quite similar to those reported by Fry *et al.*²⁶ and Don *et al.*,²⁷ who found it in about 12% of children with CAP in, respectively, Thailand and Italy. However, as observed in other studies,^{28–30} it was mainly found in association with other viruses that are often detected singly (particularly RSV and rhinovirus). This suggests that bocavirus may not play a primary role as a cause of CAP, and that its presence in respiratory secretions is owing to a previous infection followed by prolonged low-level viral shedding. Furthermore, in a previous study of children with respiratory diseases and bocavirus infection, we found that the most severe clinical pictures were diagnosed in children suffering from co-infections, whereas most of the cases associated with a single bocavirus infection were very mild.³¹

The role played by hMPV seems to be more important. Its overall frequency was quite similar to that of bocavirus, but it was detected in association with other viruses in only about 22% of the cases, which is quite similar to that found by Wolf *et al.*²³ in a study carried out in Israel. This suggests that the virus may play a direct pathogenic role in the development of CAP.

Influenza viruses were the fourth most frequently detected infectious agents in children with CAP and showed the lowest incidence of co-infections. This confirms the importance of these viruses even in lower respiratory tract infections and once again underlines the need for annual vaccine administration.^{32–34}

The global incidence of infections owing to coronaviruses was 5.6%, higher than that found in a large pneumonia study carried out in Thailand.²⁶ However, the fact that all of the coronaviruses were mainly found in association with other viruses suggests that both the old and new viruses are only relatively important in pediatric CAP. Consequently, despite

Table 3. Demographic, clinical, laboratory, and radiographic variables by single viral infections

	RSV virus alone n = 112	Rhinovirus alone n = 78	Influenza virus alone n = 48	hMPV virus alone n = 36	Bocavirus alone n = 15
Demographic and clinical presentation					
Males	67 (59.8)	46 (59.0)	20 (41.7)	19 (52.8)	8 (53.3)
Mean age \pm SD, years	1.4 \pm 2.0 [^]	3.9 \pm 3.0	4.0 \pm 3.7	2.8 \pm 2.7	1.8 \pm 0.8
Presence of fever ^{**} (%)	110 (98.2)	71 (91.0)	47 (97.9)	36 (100)	14 (93.3)
High-grade fever ^{***} (%)	76 (67.9)	40 (51.3)	37 (77.1)	30 (83.3) [*]	8 (53.3)
Respiratory rate, breaths/min	60 \pm 6	58 \pm 7	55 \pm 9	57 \pm 7	56 \pm 6
SpO ₂ in room air, mean % \pm SD	91 \pm 4	91 \pm 3	93 \pm 5	92 \pm 5	93 \pm 4
Clinical findings					
Cough	88 (78.6)	58 (74.4)	39 (81.3)	26 (72.2)	13 (86.7)
Rhonchi	4 (3.6)	6 (7.7)	3 (6.3)	5 (13.9)	0
Rales	90 (80.4) [*]	52 (66.7)	36 (75.0)	31 (86.1) [*]	13 (86.7) [*]
Wheezing	30 (26.8)	22 (28.2)	9 (18.8)	11 (30.6)	8 (53.3)
Clinical outcome					
Hospitalization rate, no. (%)	65 (58.0)	46 (59.0)	24 (50.0)	17 (47.2)	11 (73.3)
Duration of hospitalization, mean days \pm SD	7.2 \pm 5.7	6.9 \pm 4.2	6.0 \pm 2.8	6.2 \pm 3.1	5.5 \pm 2.3
Drug use, no. (%)					
Antibiotics	109 (97.3)	78 (100)	44 (91.7)	36 (100)	15 (100.0)
Antipyretics	76 (79.2)	56 (80.0)	34 (82.9)	28 (77.8)	7 (63.6)
Aerosol therapy	81 (84.4)	53 (75.7)	26 (63.4)	28 (77.8)	11 (100.0)
Absence from community, mean days \pm SD	10.2 \pm 6.7	11.9 \pm 6.9	9.2 \pm 10.9	11.4 \pm 10.8	12.8 \pm 6.8
Similar illness within family	34 (30.4)	14 (18.0) [^]	26 (54.2)	16 (44.4)	1 (6.7) [^]
Laboratory data					
White blood cell count (cells/ μ l)	11 392 \pm 7652	15 545 \pm 7640	11 086 \pm 10 306	9453 \pm 5036	14 764 \pm 7059
Neutrophils (%)	50.0 \pm 18.5	64.5 \pm 18.2	48.9 \pm 22.0	49.3 \pm 15.1	62.6 \pm 16.3
Lymphocytes (%)	36.9 \pm 16.7	22.4 \pm 12.9	38.1 \pm 20.1	39.0 \pm 13.8	26.1 \pm 13.0
Monocytes (%)	12.3 \pm 4.5	10.4 \pm 5.5	11.9 \pm 4.0	10.7 \pm 3.5	9.4 \pm 4.9
Basophils (%)	0.5 \pm 0.4	0.3 \pm 0.2	0.5 \pm 0.6	0.5 \pm 0.3	0.4 \pm 0.3
Eosinophils (%)	0.4 \pm 0.6	1.1 \pm 1.7	0.6 \pm 0.8	0.6 \pm 1.2	1.5 \pm 1.2
CRP (mg/l)	25 \pm 44 [*]	71 \pm 100	24 \pm 39 [*]	23 \pm 25 [*]	17 \pm 18 [*]
Radiographic characteristics					
Non-alveolar pneumonia	61 (54.5)	37 (47.4) [^] §	35 (72.9)	27 (75.0)	7 (46.7)
Alveolar pneumonia	51 (45.5)	41 (52.6) [^] §	13 (27.1)	9 (25.0)	8 (53.3)

CRP, C-reactive protein; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; SD, standard deviation; SpO₂, peripheral oxygen saturation.

* $P < 0.05$ versus rhinovirus; [^] $P < 0.05$ versus influenza; § $P < 0.05$ versus hMPV.

**38.0°C or more at any time during the illness (before or at the time of enrolment, or during follow-up).

***39.0°C or more any time during the illness (before or at the time of enrolment, or during follow-up).

the discovery of new coronaviruses, effective preventive measures do not seem to be urgently needed. The same seems to be true in the case of enterovirus, which was found in a very small number of cases and was very frequently associated with other viruses.

Adenovirus and parainfluenza virus were identified in a very small number of cases. Previous studies have found that the frequency of identifying adenovirus can vary from 3% to 10%.⁵ Although its prevalence is always relatively low, virus should be systematically monitored because it has

been associated with severe and fatal necrotising pneumonia.¹⁷ The incidence of parainfluenza virus infection was significantly lower than that observed in previous studies,⁵ a finding that may have been at least partially due differences in the methods used to identify the viruses and the fact that we looked for a larger number of infectious agents. In most cases, they were found in association with other viruses, thus suggesting their only relative clinical importance.

Co-infections were very common, and it was not possible to define precisely the role of each pathogen as a cause

Table 4. Demographic, clinical, laboratory, and radiographic variables associated with single viral infections and co-infections

	Single virus <i>n</i> = 318	Co-infections <i>n</i> = 117	<i>P</i> value
Demographic and clinical presentation			
Males	174 (54.7)	59 (50.4)	0.41
Mean age \pm SD, years	2.7 \pm 2.7	1.7 \pm 1.4	<0.001
Presence of fever* (%)	306 (96.2)	110 (94.0)	0.32
High-grade fever** (%)	213 (67.0)	73 (62.4)	0.37
Respiratory rate, breaths/min	57 \pm 8	58 \pm 7	0.82
SpO ₂ in room air, mean % \pm SD	92 \pm 5	91 \pm 4	0.85
Clinical findings			
Cough	242 (76.1)	92 (78.6)	0.57
Rhonchi	19 (6.0)	8 (6.8)	0.74
Rales	246 (77.4)	92 (78.6)	0.78
Wheezes	88 (27.7)	32 (27.4)	0.95
Clinical outcome			
Hospitalization rate, no. (%)	181 (56.9)	59 (50.4)	0.23
Duration of hospitalization, mean days \pm SD	6.9 \pm 4.6	7.1 \pm 4.5	0.80
Drug use, no. (%)			
Antibiotics (%)	311 (97.8)	116 (99.2)	0.69
Antipyretics (%)	222 (80.4)	89 (82.4)	0.66
Aerosol therapy (%)	212 (76.8)	90 (83.3)	0.16
Absence from community, mean days \pm SD	11.1 \pm 8.4	9.7 \pm 6.1	0.42
Similar illness within the family	98 (30.8)	29 (24.8)	0.22
Laboratory data			
White blood cell count (cells/ μ l)	12 825 \pm 8050	13 707 \pm 7550	0.25
Neutrophils (%)	55.0 \pm 19.4	50.9 \pm 17.5	0.11
Lymphocytes (%)	32.4 \pm 17.0	35.9 \pm 15.2	0.09
Monocytes (%)	11.3 \pm 4.8	11.7 \pm 4.5	0.31
Basophils (%)	0.4 \pm 0.4	0.4 \pm 0.5	0.50
Eosinophils (%)	0.6 \pm 1.1	0.9 \pm 1.3	0.09
CRP (mg/l)	43 \pm 66	38 \pm 46	0.90
Radiographic characteristics			
Non-alveolar pneumonia	185 (58.2)	52 (44.4)	0.03
Alveolar pneumonia	133 (41.8)	65 (55.6)	

CRP, C-reactive protein; SD, standard deviation; SpO₂, peripheral oxygen saturation.

*38.0°C or more any time during the illness (before or at enrolment, or during follow-up).

**39.0°C or more any time during the illness (before or at enrolment, or during follow-up).

of CAP in co-infected children. However, although higher among the most frequently isolated viruses, the prevalence of co-infections was not strictly related to the absolute prevalence of the individual viruses because it was higher in the case of coronaviruses, parainfluenza viruses, enterovirus, and adenovirus than RSV, rhinovirus, and influenza viruses, even though these last were detected in significantly more children. This finding contrasts with the hypothesis of Cilla *et al.*,¹⁹ who suggested that the percentage of co-infections owing to each virus is owing to its circulation in comparison with the other viruses and seems to indicate

that other factors may play a role in favouring concomitant multiple viral infections in the same subject.

Interestingly, only marginal differences were found in the demographic, clinical, laboratory, and radiographic variables associated with the single viral infections. Although there were significant differences in the individual variables associated with a specific virus, the variability of the data makes it impossible to define a characteristic picture of the CAP cases associated with each virus. Furthermore, there were no difference between the viruses in terms of the clinical variables indicating disease severity (i.e., the rate and

duration of hospitalization and drug use), which suggests that the early use of antiviral therapy in CAP cases associated with influenza viruses should be limited in the absence of virological assays. It also highlights the importance of epidemiological surveillance and the use of molecular (or at least rapid) tests in individual patients, in order to ensure that antiviral drugs are only used in the few cases in which they are indicated.^{35,36}

Comparison of the characteristics of the single viral infections and co-infections showed that the only significant difference was the association between alveolar pneumonia and viral co-infections. As the clinical and laboratory data (including white blood cell counts and CRP concentrations) do not suggest bacterial super-infection, this finding may be explained by the synergistic role of more than one virus in causing lung inflammation and the consequent radiographic changes. On the other hand, non-alveolar CAP was similarly or more prevalent than alveolar pneumonia in the children with single viral infections.

This study has two limitations. First of all, identifying viruses in the upper respiratory secretions of children with CAP may not indicate that they are really involved in causing the disease because it may only indicate a coincidental upper airways infection or be due to a carrier state or the prolonged shedding of a pathogen that caused a previous infection. However, viruses are generally detected in no more than 5% of asymptomatic subjects, although the prevalence of rhinovirus can be as high as about 15%.¹⁹ Given the prevalence of each viral infection in our children, it is highly likely that the detected viruses played an important pathogenic role in determining CAP in most cases. However, as it has been demonstrated that viral/bacterial co-infections are common, it is possible that the CAP cases attributed to viruses by us were at least partially owing to concomitant viral and bacterial infections. The higher CRP levels and the greater frequency of alveolar CAP observed in rhinovirus-positive cases than in RSV- and influenza-positive cases may indicate a greater degree of bacterial involvement in some viral infections. We systematically performed blood cultures for all of the enrolled children in order to identify bacterial infections but, because this procedure is not very sensitive, most of the cases were negative. It is therefore not possible to draw any conclusions concerning the real incidence of viral/bacterial co-infections or to evaluate the importance of each virus in favouring bacterial super-infections. On the other hand, although it may be useful to culture sputum in order to identify respiratory bacteria responsible for CAP,³⁷ we decided not to do so because the importance of collecting sputum from children is widely debated and, even when complicated and uncomfortable procedures are used (including pharmaco-

logical pre-treatment, saline aerosol, and nasal aspiration), only some of the specimens are considered adequate.³⁸

The second limitation is related to the fact that the study was conducted in the winter and early spring, and so it may have overestimated the role of infectious agents that only circulate during this period or underestimated viruses that have a prevalent circulation in other months. Our organization in the ER does not allow active specimen collection from all CAP cases during a whole year.

In conclusion, our findings highlight the importance of respiratory viruses (mainly RSV and rhinovirus) in pediatric CAP, suggest that the only recently discovered respiratory virus frequently associated with CAP as a single infectious agent is hMPV, show marginal differences in the characteristics of the single viral infections, and demonstrate that viral co-infections are more often associated with alveolar pneumonia than single viral infections in the absence of differences in other clinical or laboratory parameters. This information is important in the management of pediatric CAP and should help to define the infectious agents that need to be prevented and for which vaccines could be developed.

Acknowledgements

This study was supported by grants from the Italian Ministry of Health (Bando Giovani Ricercatori 2007) and Amici del Bambino Malato (ABM) Onlus.

Authors' contributions

S.E. and N.P. designed the study and co-wrote the manuscript. C.D. and A.S. carried out the laboratory assays. G.P. and C.T. collected the swabs and entered the data in the database. E.F. examined the patients. I.B. evaluated the chest X-rays. C.P. statistically analyzed the data. All of the authors read and approved the final manuscript.

Competing interests

None of the authors has any competing interest to declare.

References

- 1 Heath PT. Epidemiology and bacteriology of bacterial pneumonias. *Paediatr Respir Rev* 2000; 1:4–7.
- 2 Wardlaw T, Salama P, Johansson EW, Mason E. Pneumonia: the leading killer of children. *Lancet* 2006; 368:1048–1050.
- 3 Principi N, Esposito S. Management of severe community-acquired pneumonia of children in developing and developed countries. *Thorax* 2011; 66:815–822.
- 4 Esposito S, Principi N. Emerging resistance to antibiotics against respiratory bacteria: impact on therapy of community-acquired pneumonia in children. *Drug Res Up* 2002; 5:73–87.

- 5 Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet* 2011; 377:1264–1275.
- 6 Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clin Infect Dis* 2011; 52:S284–A289.
- 7 Pneumonia Vaccine Trial Investigators' Group. Standardization of Interpretation of Chest Radiographs for the Diagnosis of Pneumonia in Children. WHO/V&B/01.35. Geneva: World Health Organization, 2001.
- 8 Walsh P, Overmyer CL, Pham K *et al.* Comparison of respiratory virus detection rates for infants and toddlers by use of flocked swabs, saline aspirates, and saline aspirates mixed in universal transport medium for room temperature storage and shipping. *J Clin Microbiol* 2008; 46:2374–2376.
- 9 Abu-Diab A, Azzeh M, Ghneim R *et al.* Comparison between per-nasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. *J Clin Microbiol* 2008; 46:2414–2417.
- 10 Lambert SB, Whiley DM, O'Neill NT *et al.* Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. *Pediatrics* 2008; 122:e615–e620.
- 11 Esposito S, Molteni CG, Daleno C *et al.* Comparison of nasopharyngeal nylon flocked swabs with universal transport medium and rayon-bud swabs with a sponge reservoir of viral transport medium in the diagnosis of paediatric influenza. *J Med Microbiol* 2010; 59(Pt 1):96–99.
- 12 Esposito S, Daleno C, Baldanti F *et al.* Viral shedding in children infected by pandemic A/H1N1/2009 influenza virus. *Virology* 2011; 8:349.
- 13 Esposito S, Indinnimeo L, Duse M *et al.* Diagnosis and treatment of community-acquired pneumonia in pediatric age –guidelines of the Italian Pediatric Societies (SIP, SITIP, SIMRI, SIAIP, SIPPS, SIMEUP). *Minerva Pediatr* 2009; 61:887–890.
- 14 Pabbaraju K, Wong S, Tokaryk KL, Fonseca K, Drews SJ. Comparison of the Luminex xTAGTM respiratory viral panel with xTAG(R) respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol* 2011; 49:1738–1744.
- 15 Gadsby NJ, Hardie A, Claas EC, Templeton KE. Comparison of the Luminex respiratory virus panel fast assay with in-house real-time PCR for respiratory viral infection diagnosis. *J Clin Microbiol* 2010; 48:2213–2216.
- 16 Lu X, Holloway B, Dare RK *et al.* Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol* 2008; 46:533–539.
- 17 Juvén T, Mertsola J, Waris M *et al.* Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 2000; 19:293–298.
- 18 Tsolia MN, Psarras S, Bossios A *et al.* Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004; 39:681–686.
- 19 Cilla G, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than three years old: high rate of viral coinfection. *J Med Virol* 2008; 80:1843–1849.
- 20 Nascimento-Carvalho CM, Ribeiro CT, Cardoso MRA *et al.* The role of respiratory viral infections among children hospitalized for community-acquired pneumonia in a developing country. *Pediatr Infect Dis J* 2008; 27:939–941.
- 21 Hamano-Hasegawa K, Morozumi M, Nakayama E *et al.* Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *J Infect Chemother* 2008; 14:424–432.
- 22 Cevey-Macherel M, Galetto-Lacour A, Gervais A *et al.* Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines. *Eur J Pediatr* 2009; 168:1429–1436.
- 23 Wolf DG, Greenberg D, Shemer-Avni Y, Givon-Lavi N, Bar-Ziv J, Dagan R. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr* 2010; 156:115–120.
- 24 Jartti T, Jartti L, Peltola V, Waris M, Ruuskanen O. Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatr Infect Dis J* 2008; 27:1103–1107.
- 25 Berkley JA, Munywoki P, Ngama M *et al.* Viral etiology of severe pneumonia among Kenyan infants and children. *JAMA* 2010; 303:2051–2057.
- 26 Fry AM, Lu X, Chittaganpitch M *et al.* Human Bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis* 2007; 195:1038–1045.
- 27 Don M, Söderlund-Venermo M, Valent F *et al.* Serologically verified human bocavirus pneumonia in children. *Pediatr Pulmonol* 2010; 45:120–126.
- 28 Allander T, Jartti T, Gupta S *et al.* Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007; 44:904–910.
- 29 Vicente D, Cilla G, Montes M, Pérez-Yarza EG, Pérez-Trallero E. Human bocavirus, a respiratory and enteric virus. *Emerg Infect Dis* 2007; 13:636–637.
- 30 Schildgen O, Müller A, Allander T *et al.* Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev* 2008; 21:291–304.
- 31 Esposito S, Bosis S, Niesters HG *et al.* Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008; 46:1337–1342.
- 32 Esposito S, Principi N. The rational use of influenza vaccines in healthy children and children with underlying conditions. *Curr Opin Infect Dis* 2009; 22:244–249.
- 33 Principi N, Esposito S, Marchisio P. Present and future of influenza prevention in pediatrics. *Expert Opin Biol Ther* 2011; 11:641–653.
- 34 Esposito S, Cantarutti L, Molteni CG *et al.* Clinical manifestations and socio-economic impact of influenza among healthy children in the community. *J Infect* 2011; 62:379–387.
- 35 Principi N, Esposito S. Antigen-based assays for the identification of influenza virus and respiratory syncytial virus: why and how to use them in pediatrics. *Clin Lab Med* 2009; 29:649–660.
- 36 Fiore AE, Fry A, Shay D *et al.* Antiviral agents for the treatment and chemoprophylaxis of influenza—recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011; 60:1–24.
- 37 Honkinen M, Lahti E, Osterback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbiol Infect* 2011; doi:10.1111/j.1469-0691.2011.03603.x. Epub Jun 14.
- 38 Lahti E, Peltola V, Waris M *et al.* Induced sputum in the diagnosis of childhood community-acquired pneumonia. *Thorax* 2009; 64:252–257.