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Respiratory viruses among children with non-severe community-acquired pneumonia: A prospective cohort study

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ABSTRACT

Background: Community-acquired pneumonia (CAP) causes a major burden to the health care system among children under-5 years worldwide. Information on respiratory viruses in non-severe CAP cases is scarce.
Objectives: To estimate the frequency of respiratory viruses among non-severe CAP cases.
Study design: Prospective study conducted in Salvador, Brazil. Out of 820 children aged 2–59 months with non-severe CAP diagnosed by pediatricians (respiratory complaints and radiographic pulmonary infiltrate/consolidation), recruited in a clinical trial (ClinicalTrials.gov Identifier NCT01200706), nasopharyngeal aspirate samples were obtained from 774 (94.4%) patients and tested for 16 respiratory viruses by PCRs.
Results: Viruses were detected in 708 (91.5%; 95%CI: 89.3–93.3) cases, out of which 491 (69.4%; 95%CI: 65.9–72.7) harbored multiple viruses. Rhinovirus (46.1%; 95%CI: 42.6–49.6), adenovirus (38.4%; 95%CI: 35.0–41.8), and enterovirus (26.5%; 95%CI: 23.5–29.7) were the most commonly found viruses. The most frequent combination comprised rhinovirus plus adenovirus. No difference was found in the frequency of RSVA (16.1% vs. 14.6%; P = 0.6), RSVB (10.9% vs. 13.2%; P = 0.4) influenza (Flu) A (6.3% vs. 5.1%; P = 0.5), FluB (4.5% vs. 1.8%; P = 0.09), parainfluenza virus (PIV) 1 (5.1% vs. 2.8%; P = 0.2), or PIV4 (7.7% vs. 4.1%; P = 0.08), when children with multiple or sole virus detection were compared. Conversely, rhinovirus, adenovirus, enterovirus, bocavirus, PIV2, PIV3, metapneumovirus, coronavirus OC43, NL63, 229E were significantly more frequent among cases with multiple virus detection.
Conclusions: Respiratory viruses were detected in over 90% of the cases, out of which 70% had multiple viruses. Several viruses are more commonly found in multiple virus detection whereas other viruses are similarly found in sole and in multiple virus detection.

1. Background

Community-acquired pneumonia (CAP) among children under-5 years old causes a major burden to health care systems worldwide [1], where it is estimated the occurrence of 156 million new CAP cases annually in this age range [2]. Therefore, effective measures to control this condition are demanded [3].

The implementation of bacteria-related vaccines, such as pneumococcal and *Haemophilus influenzae* type b conjugate vaccines, in association with the recent widespread availability of nucleic acid amplification techniques, such as real-time polymerase chain reaction (RT-PCR), had a great impact in the estimation of the proportion of respiratory virus infection in patients with acute respiratory illness [4]. However, the role of respiratory viruses remains unclear among cases with non-severe CAP, which raises concern [5]. Moreover, information on respiratory viruses in non-severe CAP cases is scarce.

2. Objectives

We estimated the frequency of the detection of respiratory viruses among cases with non-severe CAP, compared the frequency of each respiratory virus among children with sole virus detection or co-detection, and assessed age and disease duration distribution between children with or without each respiratory virus.

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3. Study design

This study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. It was approved by the Ethics Committee of the Federal University of Bahia (Approval reference number 24/2006). This was a prospective cohort study conducted at the Federal University of Bahia Hospital in Salvador, Northeast Brazil (clinical trial on the use of amoxicillin, ClinicalTrials.gov Identifier NCT01200706). From November 2006 to April 2011 community-dwelling children were seen at the Pediatric Emergency Department and diagnosed by the pediatrician on duty with non-severe CAP. CAP diagnosis was based on 1) respiratory complaints (cough or difficulty breathing) plus 2) lower respiratory pathologic findings plus 3) presence of pulmonary infiltrate or consolidation on the chest radiograph (CXR) (frontal and lateral views) taken on admission and read by the pediatrician on duty. Non-severe CAP cases were defined according to the World Health Organization criteria for severity of CAP 2000 [6]. That means, eligible patients did not present any of the following items: lower chest indrawing, inability to drink, seizure, somnolence, central cyanosis, grunting in a calm child, and nasal flaring. The exclusion criteria comprised chronic debilitating diseases (anatomic abnormalities of the respiratory tract, cancer, chronic pulmonary illness besides asthma, immunological defects, progressing neurological disorders, psychomotor retardation, heart disease with clinical repercussion, hemoglobinopathy, liver or kidney disease, severe malnutrition), other concurrent infection, HIV-infected mother, hospitalization during the previous 7 days, amoxicillin or other antibiotics use during the last 48 h, amoxicillin allergy, or history of aspiration. The primary results of the clinical trial were published in 2014 [7].

Written informed consent was collected from parents or legal guardians before enrolment. Upon screening, demographic, clinical data and nasopharyngeal aspirate samples (NPA) were collected and a complete physical examination was performed by the research team. All data were registered in pre-defined questionnaires regarding the following variables: age, sex, disease duration, complaints (cough, fever, difficulty breathing, wheezing, vomiting), axillary temperature, respiratory rate (RR), weight, findings on physical examination (chest retraction, reduced pulmonary expansion, rhonchi, wheezing, crackles). Tachypnea was considered as RR ≥ 50 breaths/min in children aged 2–11 months or RR ≥ 40 breaths/min in children from 12 to 59 months of age [8]. Nutritional evaluation was performed using the software Anthro, version 1.02 (Centre for Disease Control and Prevention and WHO) and malnutrition and severe malnutrition were defined as Z-score for weight-for-age index under −2.00 or −3.00, respectively, using the National Centre for Health Statistics standard [9].

In order to perform a post hoc analysis regarding the radiographic findings, the CXR was sent to two independent pediatric radiologists who were blinded to clinical information. Radiographic reading was entered on a standardized form according to standardized interpretation [10]. Concordant radiologically-confirmed pneumonia was identified if there was agreement on the presence of pulmonary infiltrate or consolidation in the independent assessment by two radiologists. If there was disagreement between the initial radiologists, CXR was sent to a third pediatric radiologist who used the same methods. CXR reading was finally defined as agreed or not by two radiologists. All radiologists have worked primarily in pediatric radiology post completion of a two-year residency, with twenty, twenty-five, and thirty years of experience.

Immediately after collection, the NPA samples were stored at −80 °C at the Federal University of Bahia Hospital Laboratory until shipment to the University of Turku Clinical Virology Department, Turku, Finland, by airplane at −80 °C in dry ice. After thawing, a multiplex real-time PCR test kit (Anyplex [TM] II RV16, Seegene, Seoul, South Korea) [11] was performed to detect the following viruses: human adenovirus (HAdV), influenza A (Flu A) and B (Flu B) viruses, parainfluenza virus types 1–4 (PIV 1, PIV 2, PIV 3, PIV 4), rhinovirus

Table 1
Baseline characteristics of children with non-severe community-acquired pneumonia.

Characteristics	All Cases (n = 774) n (%)[a]	Concordant radiologically-confirmed pneumonia cases (n = 272) n (%)[a]
Demographics		
Age (median [IQR] months)	25.5 (14.1–40.0)	30.8 (18.3–44.4)
Male gender	411 (53.1)	141 (51.8)
History		
Disease duration(median [IQR] days)	5 (4–8)	7 (4–10)
Cough	754/772[b] (97.7)	268/271[c] (98.9)
Fever	715/773[b] (92.5)	262 (96.3)
Difficulty breathing	484/772[b] (62.7)	182/271[c] (67.2)
Vomiting	346/773[b] (44.8)	119 (43.8)
Physical examination		
Rhonchi	503/773[b] (65.1)	183 (67.3)
Crackles	349/773[b] (45.1)	151 (55.5)
Tachypnea	346/773[b] (44.8)	141 (51.8)
Wheezing	228/773[b] (29.5)	68 (25.0)
Reduced pulmonary expansion	66/772[b] (8.5)	45/271[c] (16.6)
Malnutrition	29/773[b] (3.8)	7 (2.6)
Chest retraction	28/773[b] (3.6)	13 (4.8)

IQR, interquartile range.
[a] Expressed as absolute number and percentage if not otherwise specified.
[b] The denominator was not 774 because there was missing information.
[c] The denominator was not 272 because there was missing information.

Table 2
Frequency of respiratory viruses detected among children with non-severe CAP.

	All cases n = 774	Concordant radiologically-confirmed pneumonia cases n = 272
Viruses	n (%; 95% CI)	n (%; 95% CI)
Rhinovirus	357 (46.1; 42.6–49.6)	128 (47.1;41.2–53.0)
Adenovirus	297 (38.4; 35.0–41.8)	110 (40.4;34.7–46.4)
Enterovirus	205 (26.5; 23.5–29.7)	85 (31.3;26.0–36.9)
Respiratory Syncytial Viruses	193 (24.9; 22.0–28.1)	54 (19.9;15.4–24.9)
RSV A	109/763[a] (14.3; 11.9–16.9)	35/267[b] (13.1;9.4–17.6)
RSV B	81/763[a] (10.6; 8.6–13.0)	16/267[b] (6.0;3.6–9.3)
Bocavirus	174 (22.5; 19.6–25.5)	72 (26.5;21.5–32.0)
Parainfluenza viruses	159 (20.5; 17.8–23.5)	65 (23.9;19.1–29.2)
PIV 1	31 (4.0; 2.8–5.6)	11 (4.0;2.1–6.9)
PIV 2	28 (3.6; 2.5–5.1)	9 (3.3;1.6–6.0)
PIV 3	68 (8.8; 6.9–10.9)	29 (10.7;7.4–14.8)
PIV 4	47 (6.1; 4.5–7.9)	20 (7.4;4.7–10.9)
Metapneumovirus	100 (12.9; 10.7–15.4)	31 (11.4;8.0–15.6)
Influenza viruses	66 (8.5; 6.7–10.7)	15 (5.5;3.2–8.7)
Flu A	42 (5.4; 4.0–7.2)	9 (3.3;1.6–6.0)
Flu B	26 (3.4; 2.3–4.8)	6 (2.2;0.9–4.5)
Coronaviruses	64 (8.3; 6.5–10.4)	16 (5.9;3.5–9.2)
OC43	43 (5.6; 4.1–7.3)	12 (4.4;2.4–7.4)
NL63	16 (2.1; 1.2–3.3)	3 (1.1;0.3–3.0)
229E	13 (1.7; 0.9–2.8)	2 (0.7;0.1–2.4)

CAP, community-acquired pneumonia; CI, confidence interval.
[a] The denominator was not 774 because there was missing information.
[b] The denominator was not 272 because there was missing information.