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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.

Table 3
Comparison of the frequency of each respiratory virus in children with sole or multiple virus detection among children with non-severe CAP and virus detected.

Respiratory Viruses	All cases			Concordant radiologically-confirmed pneumonia cases		
	Detection		P	Detection		P
	Multiple (n = 491)	Sole (n = 217)		Multiple (n = 170)	Sole (n = 68)	
Rhinovirus	302 (61.5)	55 (25.3)	< 0.001	110 (64.7)	18 (26.5)	< 0.001
Adenovirus	272 (55.4)	25 (11.5)	< 0.001	100 (58.8)	10 (14.7)	< 0.001
Enterovirus	201 (40.9)	4 (1.8)	< 0.001	83 (48.8)	2 (2.9)	< 0.001
Respiratory Syncytial Viruses	131 (26.7)	62 (28.6)	0.6	41 (24.1)	13 (19.1)	0.4
RSV A	78/485 (16.1)[a]	31/212 (14.6)[a]	0.6	29/167 (17.4)[a]	6/66 (9.1)[a]	0.1
RSV B	53/485 (10.9)[a]	28/212 (13.2)[a]	0.4	10/167 (6.0)[a]	6/66 (9.1)[a]	0.4
Bocavirus	165 (33.6)	9 (4.1)	< 0.001	68 (40.0)	4 (5.9)	< 0.001
Parainfluenza viruses	134 (27.3)	25 (11.5)	< 0.001	57 (33.5)	8 (11.8)	0.001
PIV 1	25 (5.1)	6 (2.8)	0.2	9 (5.3)	2 (2.9)	0.7
PIV 2	26 (5.3)	2 (0.9)	0.006	9 (5.3)	0	0.06
PIV 3	60 (12.2)	8 (3.7)	< 0.001	26 (15.3)	3 (4.4)	0.02
PIV 4	38 (7.7)	9 (4.1)	0.08	17 (10.0)	3 (4.4)	0.2
Metapneumovirus	81 (16.5)	19 (8.8)	0.006	24 (14.1)	7 (10.3)	0.4
Influenza viruses	51 (10.4)	15 (6.9)	0.1	12 (7.1)	3 (4.4)	0.6
Flu A	31 (6.3)	11 (5.1)	0.5	6 (3.5)	3 (4.4)	0.7
Flu B	22 (4.5)	4 (1.8)	0.09	6 (3.5)	0	0.2
Coronaviruses	61 (12.4)	3 (1.4)	< 0.001	13 (7.6)	3 (4.4)	0.6
OC43	41 (8.4)	2 (0.9)	< 0.001	10 (5.9)	2 (2.9)	0.5
NL63	15 (3.1)	1 (0.5)	0.03	2 (1.2)	1 (1.5)	1
229E	13 (2.6)	–	0.01	2 (1.2)	0	1

[a] Different denominator from the whole subgroup due to missing data on RSV subtype.

(RV), respiratory syncytial viruses A (RSV A) and B (RSV B), human bocavirus 1 (HBoV1), coronaviruses 229E (HCoV 229E), NL63 (HCoV NL63), and OC43 (HCoV OC43), metapneumovirus (hMPV), and enteroviruses (EV) in NPA. The Anyplex (TM) II RV16 detection kit is a multiplex real-time PCR assay based on tagging oligonucleotide cleavage extension. Moreover, an in-house triplex PCR test was used to detect RV, EV, and RSV in NPA samples, as already reported [12]. Serious measures and surveillance were performed during the whole period of laboratory procedures in order to avoid PCR amplicon and sample-to-sample contamination.

For the descriptive part of the study, sample size was estimated to be 384 cases considering an expected frequency of respiratory virus of 50%, total width of confidence interval (CI) of 10%, and 95% confidence interval (95% CI). For the analytical part of the study, sample size was calculated considering the smaller expected frequency of 5%, an expected difference between the compared frequencies of 5%, power of 80%, and 2-tailed test with a significance level of 0.05. Thus, the sample size was estimated as 684 cases in the study group. We performed a bivariate analysis: the frequency of each respiratory virus was compared by using chi-square or Fisher's exact test as appropriate; continuous variables were presented as median (interquartile range [IQR]) and were assessed by using Mann-Whitney *U* test due to non-parametrical distribution. As the frequency of missing information was very low, we chose to handle the missing data by excluding the cases with missing information. All analyses were performed using the Statistical Package for the Social Sciences (SPSS), Version 9.0.

4. Results

A total of 820 children were recruited in the clinical trial. Seven hundred and seventy four children (94.4%) had NPA collected. Therefore, the study group consisted of 774 cases. A subgroup of 272 (36.4%) cases had concordant radiologically-confirmed pneumonia. According to radiologists 1, 2, and 3, consolidation was found in, respectively, 84.2%, 76.8%, and 69.1% of the 272 cases with concordant radiologically-confirmed pneumonia. As per each radiologist, the other cases presented pulmonary infiltrate. Table 1 presents the baseline characteristics of the study group.

All NPA samples were tested for 16 viruses. Viruses were detected from 708 (91.5%; 95% CI: 89.3%–93.3%) cases, out of which 491 (69.4%; 95% CI:65.9%–72.7%) were positive for multiple viruses. Among 272 cases with concordant radiologically-confirmed pneumonia, respiratory virus was detected in 238 (87.5%; 95% CI:83.2%–91.0%), out of which 170 (71.4%; 95% CI:65.4%–76.9%) had multiple viruses detected. When the baseline characteristics were compared, the only difference found was in the frequency of fever, which was more common among patients with multiple viruses in regard to patients with single virus (93.9% vs. 88.4%; P = 0.01). Table 2 presents the frequency of each respiratory virus detected. RV, HAdV, and EV were the most commonly found viruses either in the whole group or in the subgroup of patients with concordant radiologically-confirmed pneumonia. The in-house triplex PCR test and Anyplex II RV16 detected RV, EV, and RSV in 77.6% and 69.9% of the NPA samples, respectively. Data on acute HBoV1 infection diagnosed by serology have been published elsewhere [13].

Overall, among 491 cases with multiple virus detection, the number of detected viruses were: 2 (n = 234; 47.7%), 3 (n = 140; 28.5%), 4 (n = 66; 13.4%), 5 (n = 34; 6.9%), 6 (n = 13; 2.6%), 7 (n = 3; 0.6%), and 8 (n = 1; 0.2%), being RV plus HAdV (n = 177; 36.0%), RV plus HBoV1 (n = 108; 22.0%), EV plus HAdV (n = 90; 18.3%), EV plus RV (n = 85; 17.3%), and HBoV1 plus HAdV (n = 42; 8.6%) the most frequent combinations, either alone or along with other viruses. On the other hand, the number of detected viruses among 170 cases with concordant radiologically-confirmed pneumonia and multiple virus detections were: 2 (n = 75; 44.1%), 3 (n = 46; 27.1%), 4 (n = 28; 16.5%), 5 (n = 14; 8.2%), 6 (n = 5; 2.9%), and 7 (n = 2; 1.2%), being RV plus HAdV (n = 49; 28.8%), EV plus RV (n = 43; 25.3%), HBoV plus HAdV (n = 37; 21.8%), RV plus HBoV (n = 32; 18.8%), and EV plus HAdV (n = 30; 17.6%) the most frequent combinations, either alone or along with other viruses too.

Table 3 shows the comparison of the frequency of each virus among children with sole or multiple virus detection. In the whole group, no difference was found in the frequency of RSV A, RSV B, Flu A, Flu B, PIV 1, or PIV 4. Among 238 cases of non-severe CAP with concordant radiologically-confirmed pneumonia and virus detected, RV, HAdV, EV, HBoV1, and PIV 3 were significantly more frequent among cases with detection of multiple viruses. Table 4 compares the distribution of age and disease duration between children with or without each respiratory

Table 4
Comparison of age and disease duration among children with non-severe CAP with or without each respiratory virus.

Respiratory virus	Median (IQR) age (months)			Median (IQR) disease duration (days)		
	Virus detected			Virus detected		
	Yes	No	P	Yes	No	P
Rhinovirus WG	26.6 (14.1; 41.3)	24.1 (14.0; 39.1)	0.2	5 (3; 10)	5 (4; 8)	0.7
CRCPSG	32.4 (19.3; 45.9)	28.8 (17.7; 42.8)	0.3	7 (4; 12)	7 (4; 10)	0.6
Adenovirus WG	25.8 (15.1; 41.2)	25.2 (13.5; 39.3)	0.2	5 (3; 10)	5.5 (4; 8)	0.9
CRCPSG	30.3 (19.1; 43.0)	31.0 (16.7; 46.1)	0.9	7 (4; 14)	7 (4;10)	0.8
Enterovirus WG	25.3 (15.4; 39.4)	25.5 (13.6; 40.2)	0.7	5 (4; 8)	5 (4; 8)	0.8
CRCPSG	32.2 (17.6; 43.6)	30.3 (18.4; 45.8)	0.7	7 (4; 11)	7 (4; 10)	0.6
Respiratory Syncytial Viruses WG	24.0 (13.6; 35.6)	26.4 (14.3; 42.0)	0.02	5 (3; 7)	6 (4; 10)	< 0.001
CRCPSG	25.7 (15.9; 39.3)	31.6 (18.9; 45.9)	0.1	5 (4; 7)	7 (4; 11)	0.01
RSV A WG	21.9 (13.5; 35.3)	26.3 (14.3; 41.2)	0.02	5 (3; 7)	6 (4; 9)	0.002
CRCPSG	24.3 (16.2; 40.9)	31.2 (18.6; 44.8)	0.3	5 (4; 7)	7 (4; 10)	0.04
RSV B WG	25.2 (14.1; 36.0)	25.6 (14.1; 40.9)	0.5	5 (3; 7)	6 (4; 8)	0.005
CRCPSG	25.7 (17.0; 33.9)	31.2 (18.4; 44.9)	0.2	5 (3; 7)	7 (4; 10)	0.1
Bocavirus WG	26.1 (17.4; 38.1)	25.1 (13.5; 40.8)	0.4	6 (4; 11)	5 (4; 8)	0.1
CRCPSG	26.8 (21.0; 40.0)	31.3 (16.5; 46.2)	0.6	7 (4; 15)	7 (4; 10)	0.3
Parainfluenza viruses WG	26.7 (14.3; 42.0)	25.2 (13.8; 39.3)	0.5	6 (4; 8)	5 (4; 8)	0.6
CRCPSG	32.5 (14.2; 46.3)	30.3 (19.7; 44.0)	0.8	7 (5; 10)	7 (4; 10)	0.9
PIV 1 WG	39.1 (14.3; 44.2)	25.2 (14.0; 39.6)	0.1	6 (3; 7)	5 (4; 8)	0.5
CRCPSG	40.9 (12.4; 50.3)	30.7 (18.4; 44.2)	0.8	7 (5; 7)	7 (4; 10)	0.6
PIV 2 WG	34.3 (19.4; 42.3)	25.1 (13.8; 39.8)	0.1	5 (3; 7)	5 (4; 8)	0.4
CRCPSG	36.0 (12.1; 41.9)	30.7 (18.4; 44.8)	0.6	5 (4; 11)	7 (4; 10)	0.4
PIV 3 WG	18.7 (11.4; 30.8)	26.3 (14.3; 40.4)	0.01	6 (3.25; 10)	5 (4; 8)	0.5
CRCPSG	22.6 (11.0; 44.6)	31.2 (19.2; 44.4)	0.2	7 (5; 11)	7 (4; 10)	0.9
PIV 4 WG	36.2 (15.2; 46.3)	24.9 (13.9; 39.4)	0.04	7 (5; 8)	5 (4; 8)	0.08
CRCPSG	41.0 (24.5; 52.7)	29.4 (18.1; 43.8)	0.04	7 (5; 10)	7 (4; 10)	0.5
Metapneumovirus WG	24.7 (13.3; 37.9)	25.8 (14.1; 40.1)	0.8	6 (4; 8)	5 (4; 8)	0.9
CRCPSG	31.4 (22.7-50.4)	30.7 (17.5; 44.3)	0.2	7 (4; 10)	7 (4; 10)	0.9
Influenza viruses WG	27.0 (14.8; 37.8)	25.1 (13.9; 40.2)	0.6	5 (4; 7.25)	5 (4; 8)	0.5
CRCPSG	21.1 (11.4; 34.4)	31.2 (18.4; 44.7)	0.1	8 (7; 10)	7 (4; 10)	0.2
Flu A WG	28.8 (14.8; 46.0)	25.1 (13.9; 40.0)	0.3	5 (3; 7.25)	5 (4; 8)	0.3
CRCPSG	23.7 (13.0; 34.6)	30.9 (18.4; 44.6)	0.3	8 (7; 12)	7 (4; 10)	0.3
Flu B WG	24.6 (13.7; 37.2)	25.5 (14.1; 40.2)	0.6	5 (4; 7.5)	5 (4; 8)	0.9
CRCPSG	19.7 (8.2; 37.6)	31.0 (18.4; 44.5)	0.2	8 (5; 11)	7 (4; 10)	0.5
Coronaviruses WG	21.7 (12.3; 37.9)	26.0 (14.1; 40.1)	0.2	5 (4; 8)	5 (4; 8)	1
CRCPSG	35.9 (12.4; 44.0)	30.5 (18.3; 44.6)	0.9	7 (4; 10)	7 (4; 10)	0.9
OC43 WG	20.9 (9.3; 34.6)	26.1 (14.3; 40.3)	0.02	7 (4; 10)	5 (4; 8)	0.4
CRCPSG	35.9 (8.6; 44.0)	30.5 (18.4; 44.6)	0.7	7 (5; 10)	7 (4; 10)	0.6
NL63 WG	27.3 (15.4; 50.1)	25.5 (13.9; 39.7)	0.3	5 (3.25; 7)	5 (4; 8)	0.2
CRCPSG	41.2 (21.7; 56.0)	30.7 (18.2; 44.4)	0.4	3 (1; 10)	7 (4; 10)	0.2
229E WG	29.7 (14.9; 47.5)	25.5 (14.0; 39.9)	0.6	5 (4; 8)	5 (4; 8)	0.9
CRCPSG	33.4 (22.5; 44.4)	30.8 (18.3; 44.5)	0.8	7 (4; 9)	7 (4; 10)	0.9

WG, whole group (774 cases).
CRCPSG, concordant radiologically-confirmed pneumonia subgroup (272 cases).

virus. Children with RSV A or PIV 3 or HCoV OC43 were younger than those without each one of them whereas children with PIV 4 were older than those without it. In regard to disease duration, children with RSV had a shorter disease than those without it. Similar results were found among patients with concordant radiologically-confirmed pneumonia. Table 5 presents the frequency of the baseline characteristics among patients with distinct respiratory viruses. Table 6 compares the baseline characteristics among patients with sole respiratory virus detection and detected distinct viruses. The frequency of fever, crackles and tachypnea was significantly different among the compared subgroups (Table 6).

5. Discussion

We detected respiratory viruses in 91.5% of all cases out of which multiple viruses were detected in 69.4%. RV, HAdV, and EV were the most frequently found viruses. RSV, Flu A, Flu B, PIV 1, and PIV 4 were similarly found among cases with multiple or sole detection. Children with RSV A, PIV 3, or HCoV OC43 were younger than those without each one of them whereas children with PIV 4 were older than those without it. Children with RSV had shorter disease length than those without it.

To our knowledge, our viral detection rates are the highest ones reported so far in children with CAP. A recently published study conducted in Sweden found respiratory viruses in 81% of children aged under 5 years hospitalized with CAP [14]. Another study conducted in Spain detected respiratory viruses in 83% of children aged under 18 months hospitalized with CAP [15]. Both studies employed PCR to search for the respiratory viruses in NPA: in the Swedish study, single agent and duplex real-time PCRs and in the Spanish study, multiplex PCR assay. It has been previously reported that the detection rate of singleplex PCR is higher when compared to the multiplex one showing that the singleplex PCR is more sensitive than the multiplex PCR for the specific virus [16]. However, combination of multiplex PCR tests can provide a 100% recovery rate [17]. Therefore, we attribute our high detection rate to the employment of both triplex and multiplex PCRs in this study.

The most frequently detected viruses were RV, HAdV, and EV. RV was also the most commonly detected virus among 76 children aged 6 months to 15 years hospitalized for CAP in Turku, Finland [18]. As opposed to what was pointed out by Pavia [19], EV was commonly found in our study and this can be explained by our use of two different PCR techniques to search for it. In fact, this can also explain the high rate of RV detection that, in our study, was the most frequently detected

Table 5
Frequency of baseline characteristics among patients with non-severe community-acquired pneumonia and distinct respiratory viruses.

Characteristics	Respiratory viruses detected						
	Rhinovirus n = 357	Adenovirus n = 297	Enterovirus n = 205	RSV n = 193	RSV A n = 109	RSV B n = 81	Bocavirus N = 174
Demographics							
Male gender	196 (54.9)	160 (53.9)	114 (55.6)	112 (58.0)	67 (61.5)	47 (58.0)	98 (56.3)
History							
Cough	344/356 (96.6)	286/296 (96.6)	198/204 (97.1)	191 (99.0)	108 (99.1)	80 (98.8)	170 (97.7)
Fever	316 (88.5)	277 (93.3)	194 (94.6)	183 (94.8)	102 (93.6)	78 (96.3)	166 (95.4)
Difficulty breathing	219/356 (61.5)	174/296 (58.8)	128/204 (62.7)	136 (70.5)	77 (70.6)	53 (65.4)	113 (64.9)
Vomiting	151 (42.3)	136 (45.8)	90 (43.9)	94 (48.7)	56 (51.4)	37 (45.7)	75 (43.1)
Physical examination							
Rhonchi	234 (65.5)	186 (62.6)	129 (62.9)	121 (62.7)	69 (63.3)	47 (58.0)	109 (62.6)
Crackles	156 (43.7)	125 (42.1)	98 (47.8)	95 (49.2)	55 (50.5)	35 (43.2)	83 (47.7)
Tachypnea	144 (40.3)	115 (38.7)	84 (41.0)	108 (56.0)	63 (57.8)	40 (49.4)	73 (42.0)
Wheezing	109 (30.3)	77 (25.9)	61 (29.8)	68 (35.2)	42 (38.5)	25 (30.9)	46 (26.4)
Reduced pulmonary expansion	34 (9.5)	26/296 (91.2)	20 (9.8)	9/192 (4.7)	9/108 (8.3)	0	18 (10.3)
Malnutrition	14 (3.9)	15 (5.1)	8 (3.9)	5 (2.6)	3 (2.8)	2 (2.5)	3 (1.7)
Chest retraction	12 (3.4)	12 (4.9)	10 (4.9)	8 (4.1)	6 (5.5)	3 (3.7)	6 (3.4)

Characteristics	Respiratory viruses detected					
	Parainfluenza n = 159	PIV 1 n = 31	PIV 2 n = 28	PIV 3 n = 68	PIV 4 n = 47	Metapneumovirus n = 100
Demographics						
Male gender	84 (52.8)	15 (48.4)	15 (53.6)	39 (57.4)	23 (48.9)	51 (51.0)
History						
Cough	157 (98.7)	31 (100.0)	27 (96.4)	68 (100.0)	46 (97.9)	98/99 (99.0)
Fever	149 (93.7)	30 (96.8)	28 (100.0)	64 (94.1)	42 (89.4)	96/99 (97.0)
Difficulty breathing	98 (61.6)	19 (61.3)	15 (53.6)	43 (63.2)	27 (57.4)	61/99 (61.6)
Vomiting	72 (45.3)	10 (32.3)	11 (39.3)	33 (48.5)	25 (53.2)	49/99 (49.5)
Physical examination						
Rhonchi	118 (74.2)	24 (77.4)	20 (71.4)	52 (76.5)	34 (72.3)	63/99 (63.6)
Crackles	69 (43.4)	10 (32.3)	11 (39.3)	36 (52.9)	19 (40.4)	54/99 (54.5)
Tachypnea	65 (40.9)	12 (38.7)	14 (50.0)	30 (44.1)	14 (29.8)	55/99 (55.6)
Wheezing	44 (27.7)	8 (25.8)	4 (14.3)	17 (25.0)	16 (34.0)	34/99 (34.3)
Reduced pulmonary expansion	12 (7.5)	3 (9.7)	1 (3.6)	5 (7.4)	3 (6.4)	9/99 (9.1)
Malnutrition	6 (3.8)	2 (6.5)	0	2 (2.9)	3 (6.4)	6/99 (6.1)
Chest retraction	6 (3.8)	0	0	3 (4.4)	3 (6.4)	6/99 (6.1)

Characteristics	Respiratory viruses detected						
	Influenza n = 66	Flu A n = 42	Flu B n = 26	Coronavirus n = 64	OC43 n = 43	NL63 n = 16	229E N = 13
Demographics							
Male gender	37 (56.1)	24 (57.1)	15 (57.7)	28 (43.8)	18 (41.9)	8 (50.0)	3 (23.1)
History							
Cough	61 (92.4)	38 (90.5)	25 (96.2)	61 (95.3)	42 (97.7)	15 (93.8)	12 (92.3)
Fever	64 (97.0)	41 (97.6)	24 (92.3)	59 (92.2)	42 (97.7)	14 (87.5)	11 (84.6)
Difficulty breathing	36 (54.5)	20 (47.6)	16 (61.5)	38 (59.4)	24 (55.8)	10 (62.5)	7 (53.8)
Vomiting	30 (45.5)	18 (42.9)	12 (46.2)	25 (39.1)	18 (41.9)	6 (37.5)	7 (53.8)
Physical examination							
Rhonchi	41 (62.1)	25 (59.5)	18 (30.8)	39 (60.9)	26 (60.5)	10 (62.5)	9 (69.2)
Crackles	29 (43.9)	19 (45.2)	10 (38.5)	19 (29.7)	13 (30.2)	4 (25.0)	2 (15.4)
Tachypnea	24 (36.4)	15 (35.7)	9 (34.6)	28 (43.8)	21 (48.8)	8 (50.0)	6 (46.2)
Wheezing	17 (25.8)	11 (26.2)	7 (26.9)	20 (31.3)	15 (34.9)	4 (25.0)	4 (30.8)
Reduced pulmonary expansion	4 (6.1)	3 (7.1)	1 (3.8)	3 (4.7)	3 (7.0)	0	1 (7.7)
Malnutrition	2 (3.0)	1 (2.4)	1 (3.8)	4 (6.3)	4 (9.3)	0	0
Chest retraction	2 (3.0)	2 (4.8)	0	3 (4.7)	2 (4.7)	2 (12.5)	2 (15.4)

Results are expressed as absolute number and percentage.

virus unlike previously published studies which showed RSV to have the highest detection rate [14,15]. We employed an in-house triplex PCR to search for RV, EV, and RSV besides the multiplex PCR that also investigated the presence of these three viruses along with the other 13 respiratory viruses. Notably, the pathogenic role of RV has been recently described in adults with CAP, with a more modest association in older children (5–17 years old) [20]. Additionally, RV was identified as one of the major microorganisms associated with CAP in children < 5 years of age from developing and emerging countries hospitalized with CAP in a multicenter case-control study [21].

The clinical significance of multiple respiratory virus detection causing childhood CAP is not completely clear. Recently published reports have concluded that there is no association between multiple respiratory virus detection and disease severity [22,23]. In our study, multiple viruses were detected in approximately 70% of the cases with virus-positive cases with non-severe CAP, which, to the best of our knowledge, is the highest co-detection rate reported so far. Therefore, we conclude that, mainly due to the fact that all of our cases had non-

Table 6
Comparison of baseline characteristics among patients with non-severe community-acquired pneumonia and sole virus detection.

Characteristics	Respiratory viruses detected						
	Rhinovirus n = 55	Adenovirus n = 25	Enterovirus n = 4	RSV n = 62	Bocavirus n = 9	Parainfluenza n = 25	Metapneumovirus N = 19**jha
Demographics							
Male gender	32 (58.2)	11 (44.0)	1 (25.0)	32 (51.6)	4 (44.4)	15 (60.0)	12 (63.2)
History							
Cough	53 (96.4)	23 (92.0)	4 (100.0)	62 (100.0)	9 (100.0)	25 (100.0)	18/18 (100.0)
Fever	41 (74.5)	22 (88.0)	4 (100.0)	57 (91.9)	9 (100.0)	23 (92.0)	17/18 (94.4)
Difficulty breathing	39 (70.9)	14 (56.0)	3 (75.0)	48 (77.4)	6 (66.7)	14 (56.0)	13/18 (72.2)
Vomiting	22 (40.0)	13 (52.0)	2 (50.0)	36 (58.1)	3 (33.3)	12 (48.0)	9/18 (50.0)
Physical examination							
Rhonchi	42 (76.4)	16 (64.0)	1 (25.0)	41 (66.1)	6 (66.7)	20 (80.0)	9/18 (50.0)
Crackles	27 (49.1)	5 (20.0)	3 (75.0)	34 (54.8)	6 (66.7)	5 (20.0)	14/18 (77.8)
Tachypnea	25 (45.5)	6 (24.0)	2 (50.0)	40 (64.5)	5 (55.6)	9 (36.0)	11/18 (61.1)
Wheezing	17 (30.9)	4 (16.0)	1 (25.0)	25 (40.3)	5 (55.6)	5 (20.0)	9/18 (50.0)
Reduced pulmonary expansion	7 (12.7)	3 (12.0)	0	2 (3.2)	0	1 (4.0)	3/18 (16.7)
Malnutrition	1 (1.8)	1 (4.0)	0	1 (1.6)	0	2 (8.0)	1/18 (5.6)
Chest retraction	2 (3.6)	1 (4.0)	0	3 (4.8)	1 (11.1)	2 (8.0)	1/18 (5.6)

Characteristics	Respiratory viruses detected		P
	Influenza n = 15	Coronavirus n = 3	
Demographics			
Male gender	7 (46.7)	2 (66.7)	0.8
History			
Cough	13 (86.7)	3 (100.0)	0.1
Fever	15 (100.0)	3 (100.0)	0.04
Difficulty breathing	6 (40.0)	2 (66.7)	0.2
Vomiting	5 (33.3)	1 (33.3)	0.6
Physical examination			
Rhonchi	8 (53.3)	2 (66.7)	0.2
Crackles	7 (46.7)	0	< 0.001
Tachypnea	4 (26.7)	0	0.007
Wheezing	4 (26.7)	1 (33.3)	0.2
Reduced pulmonary expansion	1 (6.7)	1 (33.3)	0.3
Malnutrition	1 (6.7)	1 (33.3)	0.2
Chest retraction	0	0	0.9

Results are expressed as absolute number and percentage.

severe CAP, viral co-detection is not related to disease severity. However, it was possible to observe that fever was significantly more frequent among cases with multiple viruses.

Recently, it was demonstrated that Flu and RSV showed a positive association with childhood CAP in a case-control study [14]. In our study, Flu A and B, RSV A and B, as well as PIV 1 and PIV 4, were similarly found in cases with sole or multiple virus detection whereas the other respiratory viruses were more common in cases with multiple virus detections (Table 3). As a matter of fact, multiple virus detection can comprise past infection, symptomatic infection, or even upcoming infection. The role of each virus found in multiple virus detection is a very complex issue. Herein, HBoV1 was more frequently found in multiple then in sole virus detection (Table 3). However, we have demonstrated that acute serologically diagnosed HBoV1 infection occurred in 22.5% of cases with multiple virus detection, as well as in 50.0% of cases with sole virus detection [13]. The challenge is to show causativeness. Effect-cause is often a problem in cross-sectional and case-control studies, especially when the predictor variable is a laboratory test; even in cohort studies, effect-cause is difficult to be shown if the disease has a long latent period [24]. In terms of lung infection like CAP, the ideal investigation should comprise lung tap [25]. Nonetheless, for ethical reasons, lung specimens are rarely obtained, so NPA are used to infer what is infecting the lung. Many of the same pathogens present in NPA can also cause CAP, might be involved in the causal chain of CAP, or might represent a concurrent upper respiratory tract infection or colonization unrelated to the pneumonic process [26]. That is why serology is a potential tool to clarify this issue [27].

In regard to age, RSVA, PIV3 and HCoV OC43 were significantly more frequent in younger patients whereas PIV4 was significantly more common in older cases (Table 4). RSV is a paramyxovirus that has been widely detected in children with CAP. The incidence and severity varies with age and younger children have been described as the more generally infected [19]. However, it has also been reported that RSV infection in younger children are more severe than in older ones [19]. Interestingly, all of our cases had non-severe CAP and RSVA was found in younger cases and in cases with shorter duration of disease. These findings demonstrate the wide presentation of RSV infection in children and may be useful in the implementation of the upcoming vaccines for the prevention of RSV infection in children [28] prioritized by age stratum. Noteworthily, all patients with enterovirus, bocavirus, influenza, or coronavirus reported fever whereas 3 quarters of the patients with rhinovirus reported it (Table 6; $P = 0.04$). Additionally, none of the patients with coronavirus had crackles nor tachypnea, whereas 77.8% and 61.1% of the patients with metapneumovirus had crackles and tachypnea, respectively (Table 6; $P < 0.001$ and 0.007, respectively). Thus, it is possible to infer that there is some variation in the presentation of the illness caused by distinct viruses.

Besides the fact that our recruitment period lasted almost 4 and a half years, reducing the chances of bias regarding viral seasonality, our

study has a major strength in enrolling a high number of cases, 820, out of which 94.4% had NPA samples collected, diagnosed by standardized criteria employed in clinical practice, increasing in this way the power and the external validity of the study. Therefore, our results may truly describe the frequency of respiratory viruses, as well as suggest the importance of each respiratory virus in children with non-severe CAP.

The main limitation of our study is the absence of healthy controls. However, respiratory virus detection has been reported in approximately 50% of asymptomatic subjects in the same age stratum as our cases [14,21]. This, in turn, reinforces the importance of respiratory viruses in children with non-severe CAP, as our detection rate was approximately 90%.

In conclusion, our study indicates that respiratory viruses are massively found in cases with non-severe CAP, being multiple viruses detected in the majority of them.

Competing interests

Authors declare they have no conflicts of interest.

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Ethical approval

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).

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