RESEARCH ARTICLE

High incidence of rhinovirus infection in children with community-acquired pneumonia from a city in the Brazilian pre-Amazon region

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Abstract

Community-acquired pneumonia (CAP) is the leading cause of child death worldwide. Viruses are the most common pathogens associated with CAP in children, but their incidence varies greatly. This study investigated the presence of respiratory syncytial virus (RSV), adenovirus, human rhinovirus (HRV), human metapneumovirus (HMPV), human coronavirus (HCoV-OC43 and HCoV-NL63), and influenza A virus (FluA) in children with CAP and the contributing risk factors. Here, children with acute respiratory infections were screened by pediatrics; and a total of 150 radiographically-confirmed CAP patients (aged 3 months to 10 years) from two clinical centers in Sao Luis, Brazil were recruited. Patient's clinical and epidemiological data were recorded. Nasopharyngeal swab and tracheal aspirate samples were collected to extract viral nucleic acid. RSV, adenovirus, rhinovirus, FluA, HMPV, HCoV-OC43, and HCoV-NL63 were detected by real-time polymerase chain reaction. The severe CAP was associated with ages between 3 and 12 months. Viruses were detected in 43% of CAP patients. Rhinovirus infections were the most frequently identified (68%). RSV, adenovirus, FluA, and coinfections were identified in 14%, 14%, 5%, and 15% of children with viral infection, respectively. Rhinovirus was associated with nonsevere CAP (*P* = .014); RSV, FluA, and coinfections were associated with severe CAP (*P* < .05). New strategies for prevention and treatment of viral respiratory infections, mainly rhinovirus and RSV infections, are necessary.

KEYWORDS
epidemiology, respiratory tract, seasonal incidence

1 | INTRODUCTION

Acute respiratory infections (ARIs) are a major cause of deaths in children worldwide, primarily in developing countries, accounting for 120 to 156 million cases annually, causing 1.4 million deaths.[1] Most of these deaths are related to the severity of community-acquired pneumonia (CAP).[2]

Children under 5 years of age are more susceptible to CAP than older children.[2] The majority (75%) of all pneumonia episodes in children under 5 years of age occur in only 15 countries, including Brazil; it is estimated that 4 million new cases are reported annually in Brazil alone.[3] What might explain higher prevalence progression to severity is the presence of additional contributing risk factors for CAP.[1] Therefore, identification of these risk factors is critical for controlling CAP, through the creation of prevention strategies.[3]

The etiology of CAP is diverse and complex; however, continuous identification of the major pathogens related to CAP is necessary for prevention and improved treatment.[4] In this context, CAP is caused by a variety of pathogenic infectious agents including viruses and bacteria, of which viruses are the most predominant in children.[5] Among viruses, respiratory syncytial virus (RSV), human rhinovirus (HRV), adenovirus (ADV), influenza A virus (FluA), human metapneumovirus (HMPV), and human coronavirus (HCoV) are the most common in children with CAP[6]; however, their frequencies vary widely.[7]

Although Brazil is listed as one of the 15 countries with the highest estimates of new cases of CAP in children worldwide,[3] few studies have investigated the viral incidence in Brazilian children with CAP. Therefore, this study identified RSV, ADV, HRV, HMPV, HCoV-OC43, HCoV-NL63, and FluA (including FluA-H1N1 subtype), as well as associated risk factors, in children with CAP from a city in the Brazilian pre-Amazon region.

2 | MATERIALS AND METHODS

2.1 | Study population

The study was conducted at Dr. Odorico de Amaral Matos Children's Hospital (with 74-bed capacity, including fourteen pediatric intensive care unit beds and six pediatric semiintensive care unit beds) and Dr. Juvêncio Mattos Children's Hospital (with 53-bed capacity, including thirty pediatric hospital bed), both academic and public hospitals serving Sao Luis, a city in the Brazilian pre-Amazon region. From November 2014 to April 2016, 150 hospital-admitted children with CAP were enrolled in the research.

The study population, which comprised patients ranging in age from 3 months to 10 years (29.7 months of average), was selected according to protocol definitions and inclusion criteria. Pneumonia cases were defined using the Brazilian Society of Pneumology and Tisiology Guideline[8] as follows: (a) presence of consolidation (a dense or fluffy opacity), pulmonary infiltrate (alveolar or interstitial densities), or pleural effusion on chest radiography; (b) two or more symptoms of acute lower respiratory tract illness, cough,

fever, difficulty breathing, age-adjusted tachypnea (≥50 breaths/min for children aged 2–11 months; ≥40 breaths/min for children aged ≥12 months) and/or wheezing. Severe pneumonia (severe CAP) was defined by the presence of lower chest concavity, inability to eat or drink, vomiting, convulsions, lethargy, unconsciousness, severe malnutrition, SpO_2 less than 90% by pulse oximetry, and/or central cyanosis.

In addition to inclusion criteria, exclusion criteria were: (a) live attenuated influenza vaccination less than 7 days before enrollment, (b) undergoing otolaryngologic surgery, (c) presence of comprised chronic debilitating disease (anatomic abnormalities of the respiratory tract, cancer, chronic pulmonary illness, asthma, immunological defects, heart disease with clinical repercussion, hemoglobinopathy, liver or kidney disease), and (d) HIV-infected mother.

2.2 | Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Ceuma University under the Certificate of Presentation for Ethical Consideration (CAAE #467.131) and was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained on the participants' behalf from their parents or legal guardians.

2.3 | Data collection

Data were recorded using questionnaires and medical records. They included age, sex, malnutrition based on the World Health Organization (WHO) criterion, type of pregnancy, low birth weight, exclusive breastfeeding for 6 months, breastfeeding for less than 12 months, completeness of immunization history, maternal education level, type of delivery, family income, numbers of smokers living in the house, and clinical data. The total seasonal cases count was calculated by mean of the number of the cases of viral infection during the period.

2.4 | Biological samples

Nasopharyngeal samples were collected from all enrolled children using a sterile swab (Plast Labor, Rio de Janeiro, Brazil). The swab was introduced into one nostril until resistance and then rotated 180°. Collected samples were stored in 2 mL of 0.9% NaCl, transported under refrigeration (4-8°C) until processing (less than 2 hours), and centrifuged at 3584g for 10 minutes. The supernatant was stored at −80°C until analysis.[9]

Tracheal aspirates were collected from all children with severe CAP by inserting a tracheal-aspiration probe as deeply as possible directly into the collection bottle in a vacuum-enclosed system (Broncozamm Tr model; Zammi Instrumental Ltd, Duque de Caxias, Brazil). Samples were sealed and transported immediately to the Laboratory of Respiratory Tract Infections at Ceuma University for processing. Samples were diluted with a mucolytic

agent (1% N-acetylcysteine, 1:1) and centrifuged at 1300*g* for 10 minutes. Supernatants were stored at −80°C until analysis.[10]

2.5 | Nucleic acid extraction and reverse transcription assay

Viral nucleic acid extraction was performed using the QIAamp MinElute virus spin kit (QIAGEN, Hilden, Germany) using 200 μL of sample, with an elution volume of 100 μL, according to the manufacturer's instructions. After extraction, concentration and purity were analyzed by spectrophotometry (Nanolite; Thermo Fisher Scientific). Complementary DNA (cDNA) synthesis was performed using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific) as previously described.[11]

2.6 | Molecular analysis

Viruses were identified by the quantitative real-time polymerase chain reaction (qPCR) using 1 μL of each primer, 1 μL of hydrolysis probe ranging from 200 to 900 nM, Ten microliters of 2× GoTaq Probe qPCR master mix (Promega), and 4 μL of cDNA in a final volume of 20 μL. qPCR assays were performed using a QuantStudio 6 Flex thermocycler (Thermo Fisher Scientific) with the program: one cycle at 95°C for 10 minutes, followed by 40 cycles at 95°C for 10 seconds and 60°C for 1 minute. Positive and negative DNA controls were used in every PCR assay to avoid false-negative and false-positive results. The primers and probes used for qPCR are listed in Table S1.

2.7 | Statistical analysis

Data were analyzed using the GraphPad Prism version 6 and Epi-Info version 6 software. Categorical variables were compared by χ^2 or Fisher's exact test. Continuous variables were compared by the Student *t* test (normal distribution) or the Mann-Whitney *U* test (without normal distribution). Statistical significance was defined as $P < .05$.

3 | RESULTS

3.1 | Epidemiological and clinical data

Among the 150 enrolled CAP patients, 23 (15%) had severe and 127 (85%) had nonsevere CAP. Most cases (90%) occurred in children with 3 to 60 months of age and all children were vaccinated with 10-valent pneumococcal conjugate vaccine and with influenza virus vaccine.

It was observed an association exists between CAP severity and low children age; among patients with severe manifestations, 74% were under 12 months old, while for nonsevere CAP manifestations, 35% presented the same range of age ($P = .002$, Figure 1).

Table 1 shows epidemiological data for the subjects according to CAP severity. In fact, CAP severity was found to be statistically
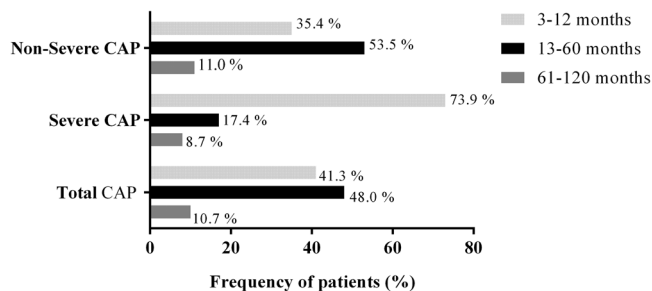

FIGURE 1 Frequency and severity of community-acquired pneumonia in association to age. CAP, community-acquired pneumonia

associated with gender. Moreover, there was a clear association between malnutrition and severe CAP, when malnourished children with severe CAP were compared with those with nonsevere CAP ($P = .065$).

The clinical data are shown in Table 2. Most of the patients presented cough (96%), dyspnea (83%), fever (83%), and wheezing (72%). In addition, 77% had less than 10 days of symptoms, 49% had tachypnea, 42% had a history of pneumonia, and 2% died.

3.2 | Viral detection

Almost half of the patients (43%) tested positive for at least one virus. HRV showed the highest detection rate (68%), followed by RSV (14%), ADV (14%), HMPV (12%), and FluA (5%). FluA-H1N1 subtype detection was negative for all patients. Moreover, a low frequency of HCoV-OC43 and HCoV-NL63 was observed in subjects (3.1% and 1.5%, respectively). Compared with severe CAP patients, HRV was statistically more detected in nonsevere CAP children ($P = .014$); while RSV and FluA infections were associated with severe CAP ($P < .0001$ and .015, respectively; Table 3).

3.3 | Coinfections and seasonality

Of the 65 patients with viral nucleic acids, 15% presented with coinfections. We observed a higher frequency of coinfections in children with severe CAP (35%) when compared with children with nonsevere CAP (4.6%, $P = .015$; Table 3).

We observed that the majority (59%) of viral infections occurred in the rainy season, with peaks between January and March (Figure 2). Moreover, all cases of RSV and FluA infections occurred during the rainy season. By contrast, rhinovirus and ADV infections were more distributed throughout the year, with higher peaks of rhinovirus between January and March.

4 | DISCUSSION

According to the World Health Organization, children under 5 years of age are more susceptible to pneumonia, with 75% of all pneumonia episodes in such children occurring in only 15 countries,

TABLE 1 Frequency and severity of community-acquired pneumonia in association with age

Variables	CAP (n = 150)	Severe CAP (n = 23)	Nonsevere CAP (n = 127)	P*
Age, media ± SD	29.7 ± 25.0	22.9 ± 33.2	30.4 ± 25.2	.341
Male gender	85 (56.7%)	19 (82.6%)	66 (52.0%)	.006
Low birth weight	22 (15.5%)[1a]	3 (14.3%)[2a]	19 (15.7%)[3a]	.999
Incomplete exclusive breastfeeding	75 (53.2%)[1b]	10 (58.8%)[2b]	65 (52.4%)[3b]	.796
Breastfeeding for <12 mo	65 (43.9%)[1c]	11 (47.8%)	54 (43.2%)[3c]	.819
Preterm birth	19 (12.8%)[1d]	2 (8.7%)	17 (13.5%)[3d]	.738
Cesarean delivery	62 (41.6%)[1d]	11 (47.8%)	51 (40.5%)[3d]	.646
Lack of prenatal treatment	22 (14.8%)[1d]	4 (17.4%)	18 (14.3%)[3d]	.749
Incomplete immunization	36 (24.3%)[1c]	4 (18.2%)[2c]	32 (25.4%)[3d]	.595
House with basic sanitation	123 (82.0%)	17 (73.9%)	106 (83.5%)	.374
School or daycare attendance	36 (24.0%)	1 (4.3%)	35 (27.6%)	.015
People per child's bedroom (>1)	120 (80.5%)[1d]	19 (82.6%)	101 (80.2%)[3d]	.999
Malnourished children	25 (20.3%)[1e]	7 (36.8%)[2d]	18 (17.3%)[3e]	.065
Severe malnutrition	8 (6.5%)[1e]	3 (15.8%)[2d]	5 (4.8%)[3e]	.639
Maternal occupation of housewife	104 (69.8%)[1d]	17 (73.9%)	87 (69.0%)[3d]	.806
Maternal education level	34 (22.8%)[1d]	5 (21.7%)	28 (22.2%)[3d]	.804
Cigarette smoker living in house	96 (64.4%)[1d]	15 (65.2%)	81 (64.3%)[3d]	.999
Low family income	108 (73.0%)[1c]	16 (72.7%)[2c]	92 (73.0%)[3d]	.999
Birth order	97 (66.0%)[1f]	17 (73.9%)	80 (63.0%)[2b]	.353

Note: Malnutrition based on the WHO criterion of a Z-score cut-off point of less than −2 standard deviations. Completeness of immunization history was defined when the child did not receive at least one vaccine. Maternal occupation was self-reported. A low maternal education level was defined as less than 9 y of schooling. A low family income was defined as less than two times the minimum wage. Values are shown as number of individuals and percentage in parentheses. Variation in sample size due to lack of data: [1a]n = 142; [1b]n = 141; [1c]n = 148; [1d]n = 149; [1e]n = 123; [1f]n = 147; [2a]n = 21; [2b]n = 17; [2c]n = 22; [2d]n = 19; [3a]n = 121; [3b]n = 124; [3c]n = 110; [3d]n = 126; [3e]n = 104.

Abbreviations: CAP, community-acquired pneumonia; SD, standard deviation.

*Significance was calculated using the χ^2 or the Fisher's exact test and compared the severe CAP vs the nonsevere CAP groups.

including Brazil.[3] In this study, it was observed that the majority of CAP cases occurred in children under 5, and that an age of less than 1 year was correlated with higher severity of CAP. In accordance with these results, Barsam et al[12] investigated the main risk factors associated with CAP in children and found that children under 5 had a higher risk for the development of CAP. Similarly, Li et al [13] investigated the incidence of CAP in Chinese children under 5 years of age, reporting that those under 1 year were more susceptible to

TABLE 2 Clinical data for children with CAP

Variables	CAP (n = 150)	Severe CAP (n = 23)	Nonsevere CAP (n = 127)	P*
Cough	114 (95.8%)[1a]	15 (100.0%)[1b]	91 (95.8%)[1c]	.844
Dyspnea	125 (83.3%)	23 (100.0%)	102 (80.3%)	.519
Fever	124 (83.2%)[2a]	18 (81.8%)[2b]	106 (83.4%)	1.000
Wheezing	108 (72.0%)	11 (47.8%)	97 (76.4%)	.265
Tachypnea	73 (48.7%)	23 (100.0%)	50 (39.4%)	.007*
Duration of symptoms				
<10 d	113 (77.4%)[3a]	15 (75.0%)[3b]	98 (77.8%)[3c]	.877
10–20 d	17 (11.6%)	3 (15.0%)	14 (11.1%)	
>20 d	16 (11.0%)	2 (10.0%)	14 (11.1%)	
History of pneumonia	62 (42.2%)[4a]	15 (71.4%)[4b]	47 (37.3%)[4c]	.107
Deaths	3 (2.0%)	3 (13.0%)	0 (0.0%)	.004*

Note: Values are shown as number of individuals and percentage in parentheses. Variation in sample size due to lack of data: [1a]n = 119; [2a]n = 149; [3a]n = 146; [4a]n = 147; [1b]n = 15; [2b]n = 22; [3b]n = 20; [4b]n = 21; [1c]n = 104; [3c]n = 126; [4c]n = 126.

Abbreviation: CAP, community-acquired pneumonia.

TABLE 3 Frequency of virus detected by qPCR in 150 children with CAP and according to CAP severity

Virus positivity	CAP, n = 65	Severe CAP, n = 17	Nonsevere CAP, n = 48	P*
Rhinovirus	44 (67.7%)	7 (41.2%)	37 (77.1%)	.014
Adenovirus	9 (13.8%)	1 (5.9%)	8 (16.6%)	.425
RSV	9 (13.8%)	9 (52.9%)	0 (0.0%)	<.0001
FluA type	3 (4.6%)	3 (17.6%)	0 (0.0%)	.015
FluA-H1N1 subtype	0 (0.0%)	0 (0.0%)	0 (0.0%)	…
HMPV	8 (12.3%)	1 (5.9%)	7 (14.5%)	.669
HCoV-OC43	2 (3.1%)	1 (5.9%)	1 (2.1%)	.457
HCoV-NL63	1 (1.5%)	0 (0.0%)	1 (2.1%)	1.000
Coinfections	10 (15.4%)	6 (35.3%)	4 (8.3%)	.015

Note: Values are shown as number of individuals and percentage in parentheses.

Abbreviations: CAP, community-acquired pneumonia; FluA, influenza A virus; HCoV, human coronavirus; HMPV, human metapneumovirus; qPCR, quantitative real-time polymerase chain reaction; RSV, respiratory syncytial virus.

*Significance was calculated using the χ^2 test or the Fisher's exact test and compared between the severe CAP group vs the nonsevere CAP group.

pneumonia. This is possibly the first study to report an association between children under 1 year of age and severe CAP. This study found an interesting gender association, with males exhibiting a higher incidence of severe CAP. However, Haugen et al[14] studied 420 children with nonsevere and severe CAP but did not observe this gender association. This disagreement suggests that new studies with higher numbers of subjects are necessary to resolve the relevance of gender as a risk factor.

The risk factors, eg, domestic environment and nutrition, are crucial to set the course of infection. However, the etiology of infection constitutes the fundamental basis of CAP development. In this study, more than 40% of all patients with CAP tested positive for at least one viral agent, with rhinovirus the most common pathogen

found. Other studies also identified rhinovirus among most frequent viruses identified in patients with ARIs, including CAP.[15,16] For example, Nascimento-Carvalho et al[17] investigated multiple respiratory viruses in 181 children with CAP from Salvador in northeastern Brazil, detecting rhinovirus most frequently. However, despite its high prevalence, few others have tested for this pathogen.[18-21] Liu et al[22] mentioned the importance of investigating the presence of rhinovirus in samples from patients with a respiratory infection, reporting that they considered including it in their study.

ADV exhibits a variable frequency in studies of patients with ARIs, including CAP. This can be explained by the variety of existing viral types and subtypes, with more than 52 ADV subtypes capable of
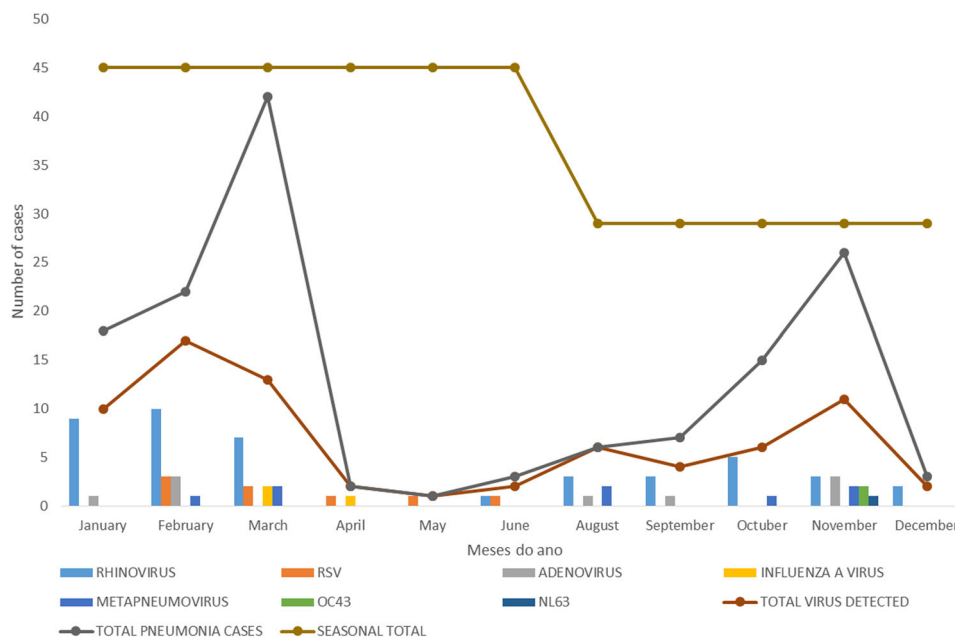

FIGURE 2 Seasonal virus detection in children with CAP. Number of cases (Y-axis) with positive results for viral pathogens: rhinovirus (blue column), RSV (red column), adenovirus (green column), and FluA virus (purple column); total virus detected (blue line); total cases of pneumonia (orange line); and total seasonal cases (black line). Seasonal totals were calculated based on the average viral detections during the seasons (rainy: January-June; dry: August-December). CAP, community-acquired pneumonia; RSV, respiratory syncytial virus

infecting humans.[23] In this study, a low frequency of ADV was observed, with most infections identified in patients with nonsevere CAP. This agrees with previous reports that also pointed to lower frequencies of ADV infection in nonsevere respiratory infections.[12,24,25] However, human ADV 55 has been responsible for large outbreaks of severe cases of pneumonia in adult patients,[26] with other studies having reported children with severe adenoviral pneumonia.[27,28] The difference between these findings might be due to viral subtypes, given that some subtypes are significantly more pathogenic than others.[29,30]

The data showed a low frequency of RSV infection; in this study, however, was observed a relationship between RSV infection and severe CAP. Interestingly, the majority of studies of children with severe CAP have reported RSV as the most common pathogen.[18,20,21,27] For example, Jonnalagadda et al[28] investigated viral etiology in 406 children with severe pneumonia and reported that RSV was the most common virus identified in 39% of subjects. In this context, the development of new vaccines and/or antivirals against RSV is crucial for prevention and treatment of RSV infections, especially in cases of CAP.

We reported that all patients positive for FluA had severe pneumonia. Several previous studies have also associated influenza virus with CAP severity, with many showing that this virus rapidly compromises lung function.[31,32] FluA has caused major epidemics and pandemics around the world, eg, in 2009, in which the H1N1 subtype was responsible for high rates of pneumonia hospitalizations.[32] However, the development of a vaccine against the most frequent influenza subtypes resulted in significant reductions of pneumonia caused by influenza worldwide.[32] This might explain the low frequency found in this study, given that Brazil makes this vaccine available to children free of charge through the Ministry of Health.

Infections with multiple viruses were associated with severe pneumonia in this study. Other studies also suggest that viral coinfection is a major contributor to severe acute respiratory syndrome.[33,34] For example, Cilla et al[34] showed a greater need for hospitalization in multiply-infected patients than for those infected with single viral infections. However, other studies have suggested that viral coinfections do not result in more severe respiratory manifestations.[35,36] Several factors including viral seasonality and pathogenicity, differences in detection methods, and viral interference might explain that conflicting information.[37] In the viral coinfections observed in our study, Rhinovirus/RSV was the most common combination. Jiang et al[38] observed similar results, showing that 48.6% of viral infections were Rhinovirus/RSV codetected infections. Likewise, Asner et al[39] showed that among 77 children with ARI and viral infection, 16.9% (12 of 77) were children with Rhinovirus/RSV codetected.

It is also necessary to highlight that the environment directly influence the severity of the manifestations, the virus species, and the coinfections. Multiple studies have reported that climatic variations influence both the etiology and frequency of CAP hospitalizations.[40,41] In this context, it is relevant to note that the city of São Luís, MA has two distinct climates: one rainy season from January to June and one dry season from July to December. In this study, was observed no seasonality for rhinovirus infections. Morikawa et al[42] primarily detected HRV type C in winter, whereas type A rhinovirus was detected throughout the year. This study did not assess all rhinovirus types, which may produce its occurrence throughout the year in the population studied.

FluA was only detected in the rain season. These data agree with the data of Nascimento-Carvalho et al[41] who found a predominance of influenza during the rainy, summer, and fall seasons, in the city of Salvador, Brazil. On the other hand, Caini et al[43] demonstrated that influenza cases peaked between June and September (dry season), in countries of the Southern hemisphere, including south and southeast Brazil. Regarding the seasonality of RSV, Bouzas et al[44] analyzed the incidence of RSV associated with seasonal distribution in the city of Salvador, Brazil. They observed the prevalence of RSV group A genotype in the dry seasons and, on the other hand, the higher incidence RSV group B genotype in the rainy season. These results agreed with ours. Understanding the seasonality associated with these pathogens is fundamental for developing seasonal strategies for prevention and treatment.[45] Currently, there are few available antiviral medications for the treatment of CAP; and only vaccines are available against influenza and ADV.[46]

In the coming future, our research will reach some potential challenges, as to include more patients, evaluate the nutritional intervention and the population education in hygiene habit; and make the molecular characterization of virus strains, besides identify the bacterial etiology. Another challenge is to include a nonpneumonia group that may help to better comprehend the respiratory infection in children as some studies have shown that rhinovirus and ADV detections are usual even among children without pneumonia.[18] It may be that these viruses cause minimal or mild disease, but depending on the subtype or the combination with bacterial or viral coinfections may result in CAP or severe CAP. This hypothesis follows observations of detection of viruses in the nasopharynx of children in the absence of clinical signs and symptoms.[47]

This is the first study conducted in a city in the Brazilian pre-Amazon region to identify viruses in children with CAP. In conclusion, such information should lead to the refinement of old, and development of new, strategies for the prevention, and treatment of viral respiratory infections.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

FUNDING INFORMATION

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REFERENCES

1. DeAntonio R, Yarzabal JP, Cruz JP, Schmidt JE, Kleijnen J. Epidemiology of community-acquired pneumonia and implications for vaccination of children living in developing and newly industrialized countries: a systematic literature review. *Hum Vaccin Immunother*. 2016;12:2422-2440.

2. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*. 2010;375:1969-1987.

3. Greenwood B. A global action plan for the prevention and control of pneumonia. *Bull World Health Organ*. 2008;86:322.

4. Huang SH, Su MC, Tien N, et al. Epidemiology of human coronavirus NL63 infection among hospitalized patients with pneumonia in Taiwan. *J Microbiol Immunol Infect*. 2017;50:763-770.

5. Sung CC, Chi H, Chiu NC, et al. Viral etiology of acute lower respiratory tract infections in hospitalized young children in Northern Taiwan. *J Microbiol Immunol Infect*. 2011;44:184-190.

6. Jain S. Epidemiology of viral pneumonia. *Clin Chest Med*. Mar 2017;38:1-9.

7. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. *J Glob Health*. 2013;3:010401.

8. Correa RA, Costa AN, Lundgren F, et al. 2018 recommendations for the management of community acquired pneumonia. *J Bras Pneumol*. 2018;44(44):405-423.

9. Brini I, Guerrero A, Hannachi N, Bouguila J, Orth-Holler D, Bouhlel A. Epidemiology and clinical profile of pathogens responsible for the hospitalization of children in Sousse area, Tunisia. *PLoS One*. 2017;12:e0188325.

10. Camargo LF, DeMarco FV, Barbas CS, Hoelz C, Bueno MA, Rodrigues M. Ventilator associated pneumonia: comparison between quantitative and qualitative cultures of tracheal aspirates. *Crit Care*. 2004;8:R422-R430.

11. Pinheiro A, Goncalves JS, Dourado AWA, et al. *Punica granatum* L. leaf extract attenuates lung inflammation in mice with acute lung injury. *J Immunol Res*. 2018;2018:6879183.

12. Barsam FJ, Borges GS, Severino AB, deMello LM, da Silva AS, Nunes AA. Factors associated with community-acquired pneumonia in hospitalised children and adolescents aged 6 months to 13 years old. *Eur J Pediatr*. 2013;172:493-499.

13. Li Y, An Z, Yin D, et al. Disease burden of community acquired pneumonia among children under 5 y old in China: a population based survey. *Hum Vaccin Immunother*. 2017;13:1681-1687.

14. Haugen J, Chandyo RK, Ulak M, et al. 25-Hydroxy-vitamin D concentration is not affected by severe or non-severe pneumonia, or inflammation, in young children. *Nutrients*. 2017;9:52.

15. Giamberardin HI, Homsani S, Bricks LF, et al. Clinical and epidemiological features of respiratory virus infections in preschool children over two consecutive influenza seasons in southern Brazil. *J Med Virol*. 2016;88:1325-1333.

16. Cebey-Lopez M, Herberg J, Pardo-Seco J, et al. Viral co-infections in pediatric patients hospitalized with lower tract acute respiratory infections. *PLoS One*. 2015;10:e0136526.

17. Nascimento-Carvalho AC, Ruuskanen O, Nascimento-Carvalho CM. Comparison of the frequency of bacterial and viral infections among children with community-acquired pneumonia hospitalized across distinct severity categories: a prospective cross-sectional study. *BMC Pediatr*. 2016;16:105.

18. Self WH, Williams DJ, Zhu Y, et al. Respiratory viral detection in children and adults: comparing asymptomatic controls and patients with community-acquired pneumonia. *J Infect Dis*. 2016;213:584-591.

19. Ali A, Khowaja AR, Bashir MZ, Aziz F, Mustafa S, Zaidi A. Role of human metapneumovirus, influenza A virus and respiratory syncytial virus in causing WHO-defined severe pneumonia in children in a developing country. *PLoS One*. 2013;8:e74756.

20. Banstola A, Banstola A. The epidemiology of hospitalization for pneumonia in children under five in the rural western region of Nepal: a descriptive study. *PLoS One*. 2013;8:e71311.

21. Berkley JA, Munywoki P, Ngama M, et al. Viral etiology of severe pneumonia among Kenyan infants and children. *JAMA*. 2010;303:2051-2057.

22. Liu WK, Liu Q, Chen DH, et al. Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study. *PLoS One*. 2014;9:e96674.

23. Holly MK, Smith JG. Adenovirus infection of human enteroids reveals interferon sensitivity and preferential infection of goblet cells. *J Virol*. 2018;92:e00250-18.

24. Homaira N, Luby SP, Petri WA, et al. Incidence of respiratory virus-associated pneumonia in urban poor young children of Dhaka, Bangladesh, 2009-2011. *PLoS One*. 2012;7:e32056.

25. Oumei H, Xuefeng W, Jianping L, et al. Etiology of community-acquired pneumonia in 1500 hospitalized children. *J Med Virol*. 2018;90:421-428.

26. Cao B, Huang GH, Pu ZH, et al. Emergence of community-acquired adenovirus type 55 as a cause of community-onset pneumonia. *Chest*. 2014;145:79-86.

27. Kim SJ, Kim K, Park SB, Hong DJ, Jhun BW. Outcomes of early administration of cidofovir in non-immunocompromised patients with severe adenovirus pneumonia. *PLoS One*. 2015;10:e0122642.

28. Jonnalagadda S, Rodriguez O, Estrella B, Sabin LL, Sempertegui F, Hamer DH. Etiology of severe pneumonia in Ecuadorian children. *PLoS One*. 2017;12:e0171687.

29. Wo Y, Lu QB, Huang DD, et al. Epidemical features of HAdV-3 and HAdV-7 in pediatric pneumonia in Chongqing, China. *Arch Virol*. 2015;160:633-638.

30. Esposito S, Zampiero A, Bianchini S, et al. Epidemiology and clinical characteristics of respiratory infections due to adenovirus in children living in Milan, Italy, during 2013 and 2014. *PLoS One*. 2016;11:e0152375.

31. Jain S, Benoit SR, Skarbinski J, Bramley AM, Finelli L. Pandemic influenza AVHIT. Influenza-associated pneumonia among hospitalized patients with 2009 pandemic influenza A (H1N1) virus–United States, 2009. *Clin Infect Dis*. 2012;54:1221-1229.

32. Thompson DS, Younger-Coleman N, Lyew-Ayee P, Greene LG, Boyne MS, Forrester TE. Influenza vaccine effectiveness in preventing influenza-associated intensive care admissions and attenuating severe disease among adults in New Zealand 2012-2015. *Vaccine*. 2018;36:5916-5925.

33. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. *Pediatr Infect Dis J*. 2005;24:605-610.

34. Cilla G, Onate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: high rate of viral coinfection. *J Med Virol*. 2008;80:1843-1849.

35. Richard N, Komurian-Pradel F, Javouhey E, et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. *Pediatr Infect Dis J*. 2008;27:213-217.

36. Paula NT, Carneiro BM, Yokosawa J, et al. Human rhinovirus in the lower respiratory tract infections of young children and the possible involvement of a secondary respiratory viral agent. *Mem Inst Oswaldo Cruz*. 2011;106:316-321.

37. Scotta MC, Chakr VC, deMoura A, et al. Respiratory viral coinfection and disease severity in children: a systematic review and meta-analysis. *J Clin Virol.* 2016;80:45-56.

38. Jiang W, Wu M, Zhou J, et al. Etiologic spectrum and occurrence of coinfections in children hospitalized with community-acquired pneumonia. *BMC Infect Dis.* 2017;17:787.

39. Asner SA, Rose W, Petrich A, Richardson S, Tran DJ. Is virus coinfection a predictor of severity in children with viral respiratory infections? *Clin Microbiol Infect.* 2015;21(264):e1-e6.

40. Lin HC, Lin CC, Chen CS, Lin HC. Seasonality of pneumonia admissions and its association with climate: an eight-year nationwide population-based study. *Chronobiol Int.* 2009;26:1647-1659.

41. Nascimento-Carvalho CM, Cardoso MR, Barral A, et al. Seasonal patterns of viral and bacterial infections among children hospitalized with community-acquired pneumonia in a tropical region. *Scand J Infect Dis.* 2010;42:839-844.

42. Morikawa S, Kohdera U, Hosaka T, et al. Seasonal variations of respiratory viruses and etiology of human rhinovirus infection in children. *J Clin Virol.* 2015;73:14-19.

43. Caini S, Andrade W, Badur S, et al. Global influenza, temporal patterns of influenza A and B in tropical and temperate countries: what are the lessons for influenza vaccination? *PLoS One.* 2016;11:e0152310.

44. Bouzas ML, Oliveira JR, Fukutani KF, et al. Acute Respiratory Infection, Wheeze Study Group Phase I, II. Respiratory syncytial virus a and b display different temporal patterns in a 4-year prospective cross-sectional study among children

with acute respiratory infection in a tropical city. *Medicine.* 2016;95:e5142.

45. Freitas AR, Donalisio MR. Respiratory syncytial virus seasonality in Brazil: implications for the immunisation policy for at-risk populations. *Mem Inst Oswaldo Cruz.* 2016;111:294-301.

46. Fraser CS, Jha A, Openshaw PJ. Vaccines in the prevention of viral pneumonia. *Clin Chest Med.* 2017;38:155-169.

47. Chonmaitree T, Alvarez-Fernandez P, Jennings K, et al. Symptomatic and asymptomatic respiratory viral infections in the first year of life: association with acute otitis media development. *Clin Infect Dis.* 2015;60:1-9.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.