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ORIGINAL ARTICLE

Is the role of rhinoviruses as causative agents of pediatric community-acquired pneumonia over-estimated?

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Abstract The role that rhinoviruses, enteroviruses, parainfluenza viruses, coronaviruses and human bocavirus play in pediatric pneumonia is insufficiently studied. We used polymerase chain reaction (PCR) to study 9 virus groups, including 16 different viruses or viral strains, in 56 ambulatory children with radiologically confirmed community-acquired pneumonia (CAP). The same tests were carried out on 474 apparently healthy control children of the same age and sex. The mean age of children with CAP was 6.5 years (SD 4.2). Respiratory syncytial virus (RSV) was found in 19.6 % of 56 cases and in 2.1 % of 474 controls. Adenoviruses were present in 12.5 % of cases (0.2 % controls) and metapneumovirus and influenza A virus each in 10.7 % of cases (0.2 % controls). Interestingly, rhinoviruses were less common in cases (10.7 %) than in controls (22.4 %): odds ratio 0.36 (95%CI) 0.15–0.87) in conditional logistic regression including 56 cases and 280 controls matched for age, sex and sampling month. The prevalence of parainfluenza viruses, enteroviruses, coronaviruses and human bocavirus were similar in both groups.

Conclusion: We conclude that the role of rhinoviruses as an etiology of pediatric CAP has been over-estimated, mainly due to the non-controlled designs of previous studies.

What is Known:
- *In non-controlled studies, rhinovirus detection has been common, next to respiratory syncytial virus, in children with viral community-acquired pneumonia (CAP).*
- *Enteroviruses, coronaviruses and the human bocavirus have been found less frequently.*

What is New:
- *In this controlled study, rhinoviruses were detected more often in healthy controls than in children with CAP, and enteroviruses, coronaviruses and human bocavirus were detected equally often in cases and controls.*
- *We conclude that previous studies have over-estimated the role of rhinoviruses in the etiology of CAP in children.*

Keywords Community-acquired pneumonia · Respiratory syncytial virus · Adenoviruses · Rhinoviruses · Human bocavirus · Child

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Abbreviations

CAP	Community-acquired pneumonia
DNA	Deoxyribonucleic acid
LRTI	Lower respiratory tract infection
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse-transcription PCR
SD	Standard deviation

Introduction

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections (LRTIs) in children, including community-acquired pneumonia (CAP). Most LRTIs caused by RSV occur in young children under 2 years of age and viral co-infections are common [2–4, 6]. The use of polymerase chain reaction (PCR) to detect respiratory viruses has revealed the role of rhinoviruses in children's LRTIs [21, 22] and has even helped to discover metapneumovirus [27] and human bocavirus [25], and rare types of coronaviruses [23, 33]. In some studies, rhinoviruses have been the second most common viruses after RSV in CAP and other LRTIs in children [3, 9] and there is increasing experimental and clinical evidence that rhinoviruses are capable of penetrating lower airways and causing invasive infections [5, 8]. Human bocavirus has been the third most common finding in children with LRTIs [3, 4] and there is preliminary evidence, based on antibody responses and results of quantitative PCR, that this virus is a real disease-causing agent [3, 9]. However, the final role of human bocavirus in pediatric respiratory infections has so far remained unresolved.

Current views on the prevalence of CAP caused by rhinoviruses are mainly based on non-controlled studies. In some studies, rhinoviruses have been identified in up to a third of children with CAP [3, 4, 9]. On the other hand, rhinoviruses have been detected with sensitive PCR techniques in respiratory samples for several weeks after acute infection [15, 24], as well as in respiratory samples of non-symptomatic children [14, 25].

Therefore, it is not enough to identify viruses in children with LRTIs. There is an urgent need for controlled studies that search for viruses in children with LRTIs compared to healthy children with no symptoms.

The aim of this study was to evaluate the viral etiology of CAP using modern PCR-based methods for 9 virus groups and 2 atypical bacteria in children treated at home and to compare the findings with healthy children.

Patients and methods

The study was performed from 1 January 2008 to 31 October 2008 and from 1 October 2010 to 31 December 2011, at the Children's City Polyclinic Number 138, a pediatric outpatient clinic in Moscow, Russia. During these study periods, 3842 children aged one to 18 years visited the outpatient clinic due to respiratory symptoms during office hours and chest radiograph was obtained in 146 cases when pneumonia was suspected. All the patients were examined by one of the authors (SK) and 102 of the children were considered to have community-acquired pneumonia (CAP) based on the clinical and radiological findings. We excluded 12 patients with bronchial obstruction on admission, 12 who had received antibiotic treatment some days before admission and 8 with severe pneumonia requiring hospitalization. Three experienced radiologists independently re-examined the chest radiographs of the remaining 70 patients, and all of them agreed with the diagnosis of radiological pneumonia in 56 cases. These 56 children aged one to 18 years with radiologically confirmed CAP who were treated at home made up the patient group.

Nasopharyngeal and oropharyngeal swab samples were available from all 56 patients and pharyngeal aspirates from 38 patients. Oropharyngeal samples were taken from the children's posterior throat using a viscose swab (300202, Deltalab, Spain), two or more hours after eating. Nasopharyngeal samples were taken through the inferior nasal meatus using a sterile flexible nasopharyngeal swab with a plastic applicator (503CS01, COPAN, Italy). Nasopharyngeal and oropharyngeal swabs from each child were combined in a single sterile tube with 0.5 ml of normal saline solution and stored at minus 70 °C until they were examined. Before we took the pharyngeal aspirate, the patients were asked to take a few consecutive deep breaths, hold their breath for a few seconds, and then forcibly exhale. A sterile catheter (Muco-Safe w. filter, Unomedica-A ConvaTec, Birkerod Co, Denmark) was then placed into the pharynx via the mouth, which caused a cough reflex, and then the airway secretions, which mainly consisted of mucus from the trachea due to coughing, were aspirated through the catheter using a suction device (Blue Cross A-750, Blue Cross Emergency Co., Ltd., Japan). The volume of the pharyngeal aspirate needed to be 1–3 ml and the specimens were stored at −70 °C until examined. Real-time reverse-transcription PCR (RT-PCR) was used to detect viruses with the Rotor-Gene 6000 Instrument (Corbett Research, Sydney, Australia), according to the manufacturer's instructions. A series of kits from the Central Research Institute for Epidemiology, Moscow, Russia, were used: the AmpliSens® Influenza virus A/B-FRT PCR kit for influenza virus A and B RNA, the AmpliSens Enterovirus-

FRT PCR kit for enterovirus RNA and the AmpliSens® ARVI-screen-FRT PCR kit for multiplex detection of respiratory syncytial virus (RSV) RNA, human metapneumovirus RNA, parainfluenzavirus type 1–4 RNA, coronavirus OC43, E229, NL63 and HKU1 RNA, rhinovirus RNA, adenovirus B, C and E DNA and human bocavirus DNA.

The DNA of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* were detected by real-time PCR with the AmpliSens® *Mycoplasma pneumoniae/Chlamydophila pneumoniae* FRT PCR kit (Central Research Institute for Epidemiology, Moscow, Russia) in all 56 nasopharyngeal and oropharyngeal swabs and in 38 pharyngeal aspirates.

The control group comprised 474 apparently healthy children of the same age and sex. The control children were examined when they attended the vaccination, or when they started the kindergarten or school. In Russia, children have to visit a pediatrician when starting kindergarten or school. Children were examined by the same pediatricians as the CAP patients, and no respiratory symptoms or signs were allowed for 2–4 weeks. Nasopharyngeal and oropharyngeal swabs, but not pharyngeal aspirates, were obtained from the controls, and RT-PCR was used to perform the same viral and bacterial examinations as were performed in the cases. The samples from the cases and controls were taken by the same doctor (SK) during the same months.

Statistics

Data analyses were performed with the software package IBM SPSS Statistics for Windows, PASW Statistics version 18.0.0 (IBM Corp, Armonk, NY). The student's *t* test and the analysis of variance were used for continuous variables and the results are expressed as means and standard deviations (SD). Fisher's exact test was used for categorized variables and the results are expressed as numbers and proportions. A p value of <0.05 between the groups was considered as statistically significant.

Conditional logistic regression analysis was used for comparison of data between 56 cases and 280 controls (five controls for each case), matched for age (<1 month difference), sex, and sampling month. A random number generator in the PASW Statistics was used in the selection of controls. The analysis was performed in the 56 sets, each consisting of one case and five controls. Results of conditional logistic regression analysis are presented as adjusted p value, odds ratios (OR) and 95 % confidence intervals (95%CI).

Results

The mean age of 56 children with CAP was 6.5 years (SD 4.2 years, range 1–16.8 years, median 5.25 years), compared to 6.2 years (3.2, 1–17.5, 5.75) in 474 controls. Over half of CAP patients (58.9 %) were less than 5 years old, compared to 41.1 % of controls. The proportion of boys was 53.6 % in cases and 52.3 % in controls.

At least one virus was detected in respiratory samples in 36 (64.3 %) of 56 cases and in 166 (35.2 %) of 474 controls ($p < 0.001$). Multiple viruses were detected in 7 CAP patients (19.4 %): RSV plus adenovirus in 4 cases and influenza A virus, metapneumovirus, coronavirus and human bocavirus in others. Only swabs were obtained from 18 patients and 13 (72.2 %) were virus-positive. Both swabs and aspirates were obtained from 38 patients and 30 (78.9 %) were virus-positive; both samples in 16 (53.3 %) cases, only swabs in 4 (13.3 %) cases and only aspirates in 10 (33.3 %) cases. Thus, pharyngeal aspirates were positive in 26/38 (68.4 %) and nasopharyngeal and/or oropharyngeal swabs in 33/56 (58.9 %) cases.

The most common viruses in CAP were RSV, which was present in 19.6 % of cases (30.6 % of all virus positive), and adenoviruses, which were present in 12.5 % of cases (Table 1). Metapneumovirus, influenza A virus and rhinoviruses were each found in 10.7 % (Table 1).

RSV (2.1 %), metapneumovirus (<1 %), influenza A virus (<1 %) and adenoviruses (<1 %) were rarely found in controls (Table 1). Rhinoviruses were found more often in controls (22.4 %, adjusted $p = 0.022$) than in cases (10.7 %) (Table 1).

Parainfluenza viruses were found in 3.6 % of cases and in 5.5 % of controls, and coronaviruses in 5.4 and 3.4 %, respectively (Table 1). Enteroviruses and human bocavirus were found only occasionally (Table 1).

The 56 cases and 280 controls matched for age, sex and sampling months were included in the conditional logistic regression analyses, and RSV (OR 7.7), metapneumovirus (OR 21.1), influenza A virus (OR 16.4) and adenoviruses (OR 15.5) were more common, but rhinoviruses were less common (OR 0.36, 95%CI 0.15–0.87), in CAP cases than in controls (Table 1).

RSV, influenza A virus and adenoviruses were predominantly detected in children aged less than 5 years (Table 2). Rhinoviruses were found in 28.0 % of children aged 3 to 5 years compared to 16.0 % in younger children ($p = 0.038$) and parainfluenza viruses were found in 9.1 and 3.1 %, respectively ($p = 0.025$).

The presence of at least one virus was highest in children aged 3 to 5 years (61.4 %) compared to 44.7 % of those aged 1 to 3 ($p = 0.015$), 33.3 % of those aged 6 to 10 ($p < 0.001$) and 31.6 % of those aged 10 to 17 ($p < 0.0001$).

No viruses were found in 24 control samples obtained in summer, but positive results were obtained for 45.9 % of those

Table 1 Viral findings in 56 children with community-acquired pneumonia and in 474 healthy controls

Virus	CAP patients (swabs and aspirates) (n = 56)	CAP patients (swabs only) (n = 56)	Controls (n = 474) Age- and sex-matched controls (n = 280)	p[a] p[b]	Odds ratio[c] (95 % CI)
RSV	11 (19.6 %)	7 (12.5 %)	10 (2.1 %)	<0.001	
			5 (1.8 %)	0.001	7.71 (2.33–25.89)
Human meta-pneumovirus	6 (10.7 %)	4 (7.1 %)	1 (0.2 %)	<0.001	
			1 (0.4 %)	0.003	21.08 (2.31–192.28)
Influenza A virus	6 (10.7 %)	6 (10.7 %)	3 (0.6 %)	<0.001	
			2 (0.7 %)	<0.001	16.38 (3.21–83.47)
Adenovirus	7 (12.5 %)	3 (5.4 %)	1 (0.2 %)	<0.001	
			1 (0.4 %)	0.016	15.51 (1.58–151.97)
Rhinovirus	6 (10.7 %)	6 (10.7 %)	106 (22.4 %)	0.055	
			69 (24.6 %)	0.022	0.36 (0.15–0.87)
Parainfluenza virus	2[d] (3.6 %)	2[d] (3.6 %)	25[e] (5.3 %)	0.757	
			22 (7.9 %)	0.395	0.43 (0.097–1.87)
Coronavirus	3 (5.4 %)	3 (5.4 %)	16 (3.4 %)	0.440	
			6 (7.5 %)	0.182	2.54 (0.62–10.47)
Enterovirus	1 (1.8 %)	1 (1.8 %)	11 (2.3 %)	1.000	
			9 (11.3 %)	1.000	0.54 (0.067–4.33)
Human bocavirus	1 (1.8 %)	1 (1.8 %)	7 (1.5 %)	0.593	
			7 (2.5 %)	1.000	0.696 (0.084–5.77)

[a] In comparison to all controls

[b] CAP patients (swabs) in comparison to age and sex-matched controls

[c] Conditional logistic regression analysis including 56 cases (swabs) and 280 age-and sex-matched controls

[d] Type 2 in 1 case, type 3 in 1 case

[e] Type 1 in 4, type 2 in 15, type 3 in 3, and type 4 in 3 controls

148 obtained in autumn, 59.0 % of those 39 obtained in winter and 32.7 % of those 263 obtained in spring (Table 3). Rhinoviruses were common in spring (25.9 % positive) and autumn (21.6 % positive) (Table 3).

PCR was positive for *Mycoplasma pneumoniae* in 12 (21.4 %) children with CAP and in none of controls ($P < 0.001$). It was the only identified agent in 9 (75 %) cases. *Mycoplasma* was identified in 2 (6.8 %) 1 to 5 years old, in 7 (38.9 %) 6 to 10 years old and in 3 (33.3 %) 11 to 17 years old CAP patients.

PCR was positive for *Chlamydophila pneumoniae* in only 3 (5.4 %) children with CAP; one case was in 6 to 10 years old

Table 2 Viral findings in relation to age, presented as combined figures for the 36 virus-positive pneumonia patients and 166 virus-positive controls

Virus	Age 1–2 years (n = 94)	Age 3–5 years (n = 132)	Age 6–10 years (n = 225)	Age 11–16 years (n = 79)
RSV (n = 21)	4 (4.3 %)	9 (6.8 %)	6 (2.7 %)	2 (2.5 %)
Human meta-pneumovirus (n = 7)	2 (2.1 %)	4 (3.0 %)	1 (0.4 %)	0
Influenza A virus (n = 9)	3 (3.2 %)	4 (3.0 %)	1 (0.4 %)	1 (1.3 %)
Adenovirus (n = 8)	4 (4.3 %)	3 (2.3 %)	1 (0.4 %)	0
Rhinovirus (n = 112)	15 (16.0 %)	37 (28.0 %)	46 (20.4 %)	14 (17.7 %)
Parainfluenza virus (n = 27)	4 (4.3 %)	12 (9.1 %)	7 (3.1 %)	4 (5.1 %)
Coronavirus (n = 19)	4 (4.3 %)	1 (0.8 %)	11 (4.9 %)	3 (3.8 %)
Enterovirus (n = 12)	4 (4.3 %)	6 (4.5 %)	2 (0.9 %)	0
Human bocavirus (n = 8)	2 (2.1 %)	5 (3.8 %)	0	1 (1.3 %)
All viruses (n = 223)	42 (44.7 %)	81 (61.4 %)	75 (33.3 %)	25 (31.6 %)

Table 3 Rhinovirus, parainfluenza virus, coronavirus, enterovirus and human bocavirus findings in 474 controls in relation to the season

Season	Rhino-viruses (n = 106)	Parainfluenza viruses (n = 25)	Coronavirus (n = 16)	Enterovirus (n = 10)	Human bocavirus (n = 7)	All viruses (n = 180)
Winter (December) (n = 39)	6 (15.4 %)	6 (15.4 %)	1 (2.3 %)	1 (2.3 %)	1 (2.3 %)	23 (59.0 %)
Spring (March, May) (n = 263)	68 (25.9 %)	5 (1.9 %)	9 (3.4 %)	0	4 (1.5 %)	86 (32.7 %)
Summer (July, August) (n = 24)	0	0	0	0	0	0
Autumn (September, October, November) (n = 148)	32 (21.6 %)	14 (9.5 %)	6 (4.1 %)	9 (6.1 %)	2 (1.4 %)	68 (45.9 %)

and two cases in 11 to 17 years old. *Chlamydophila* was found in 3 (0.6 %) non-symptomatic controls, all being more than 6 years old.

Discussion

There are three main results in this study on children with CAP treated at home. Firstly, RSV, metapneumovirus, influenza A virus and adenoviruses were significantly more common in children with CAP than in controls. This finding fits well with the established evidence that these viruses are important CAP causing agents in children. Secondly, we found equal incidences of parainfluenza viruses, coronaviruses, enteroviruses and human bocavirus in cases and controls. Thirdly, although rhinoviruses were quite frequently (10.7 %) identified in CAP patients, the rate was as high as 22.4 % in non-symptomatic controls. This finding that rhinoviruses were less common in CAP cases than in controls matched for age, sex and month of sampling was confirmed with conditional logistic regression. Our current results suggest that the role of rhinoviruses in children's LRTIs is not as important as estimated in previous non-controlled studies [3, 4, 19, 27].

RSV was found in almost 20 % of children with CAP, while metapneumovirus, influenza A virus and adenoviruses were found in about 10 %. These figures agree well with earlier observations [3, 4, 21, 23], even though there have been considerable variations, according to ages of patients and epidemiological situations. We only enrolled non-hospitalized children aged over 12 months, and the incidence of RSV is highest among children aged less than 12 months [16, 19].

During the surveillance periods, low levels of activity for RSV circulation were recorded in Moscow. In March 2009, the Central Research Institute for Epidemiology, Moscow, Russia, reported that RSV only occurred in 13 % of children hospitalized with acute respiratory infections [10] and in February 2011 just in 4.8 % [11]. Influenza activity was also low during the 2008–2009 season, but was high during the 2010–2011 season. In February 2009, influenza A virus occurred in 7 % [10], but in February 2011 in 30 %, of children hospitalized with acute respiratory infections [11].

The use of PCR for respiratory infections has changed our knowledge on the carriage of respiratory viruses, as well as on the frequency of multiple viral infections. There may be temporary, short-time carriages of RSV, influenza A and B viruses and metapneumovirus during epidemics [32]. Adenoviruses may cause silent chronic infections with occasional acute exacerbations [7]. Mixed viral-bacterial respiratory infections are common [2, 9, 12, 16], but our study design did not allow to separate viral cases from mixed viral-bacterial cases.

Recent studies have highlighted the role of rhinoviruses in the etiology of LRTI in children, being identified in up to 30 % of children with CAP [3, 9, 16]. On the other hand, sensitive PCR techniques can identify rhinoviruses in respiratory samples for several weeks after acute infection [15, 24], as well as in non-symptomatic children who have not reported any recent or subsequent respiratory infections [14, 25]. There is evidence that rhinoviruses are able to replicate in human lower airway cells [28] and may be associated with lower respiratory tract infections [4, 8, 12]. Since there are more than 100 rhinovirus serotypes, the development of antibody assays for rhinoviruses has not been successful [15]. Thus, the final role of rhinoviruses in children's CAP has remained unresolved. Blood myxovirus resistance protein A (MxA) level is increased in children with symptomatic respiratory viral infections, including rhinovirus infections, and is a promising method to separate carriers from those with infection [30]. Our finding that rhinoviruses were more common (22.4 %) in healthy controls than in CAP patients suggests that the role of rhinoviruses has been over-estimated in previous, non-

controlled, PCR-based studies, and larger, prospective, controlled studies, as well as studies applying MxA or other virus-specific host markers, are needed.

Studies that use PCR for respiratory samples have identified human bocavirus as the third most common virus in children with different respiratory infections [3, 9]. Some studies have reported antibody responses to human bocavirus [17, 31] and some using quantitative PCR have highlighted the association between symptom severity and numbers of viral copies in respiratory samples [35]. Thus, human bocavirus is capable of causing clinical infections of the lower airways, but it is not known how often. In addition, the majority of human bocavirus findings have been mixed findings with other viruses [3, 4]. Thus, the role of human bocavirus as a causative agent in children's respiratory infections is still unresolved and our findings suggest that the role is minor in pediatric CAP.

Mycoplasma etiology of CAP was defined in 21.4 % of our patients and *Chlamydophila* etiology in 5.4 %. Previous studies have reported conflicting results. In a Swedish study, *Mycoplasma pneumoniae* was identified by PCR in 29.0 % of children with respiratory symptoms, and only in 0.4 % of apparently healthy children [20], whereas in a Dutch study, the figures were 21.2 % in non-symptomatic and 16.2 % in symptomatic children [29]. Neither serology nor quantitative PCR nor culture differentiated non-symptomatic carrier from infection [29]. The absence of *Mycoplasma pneumoniae* findings and the rarity of *Chlamydophila pneumoniae* findings (0.6 %) in healthy controls in the present study suggest that these two atypical bacteria had some role in the etiology of CAP in children.

There are three strengths in the present study. Firstly, the study was prospective and included healthy, non-symptomatic controls. Secondly, all CAP cases were verified both clinically and radiologically. Thirdly, a large PCR-based test panel that covered 9 virus groups and 16 viruses or virus types and 2 atypical bacteria was available. In particular, we covered rhinoviruses, enteroviruses, coronaviruses and human bocavirus, which are common in respiratory samples but whose role in pediatric pneumonia is still uncertain. The minor role of rhinoviruses in pediatric CAP was confirmed with conditional logistic regression by comparing the cases with controls matched for age, sex and sampling month.

The most important shortcoming of the study was the small number, 56, of CAP cases and the large age distribution. However, as many as 474 controls were included and viral findings were established in 180 of them and 280 controls were included in conditional logistic regression analysis. In addition, our findings were clear, since RSV, influenza A virus, metapneumovirus and adenoviruses were only found in occasional controls, and coronaviruses, human bocavirus and parainfluenza viruses were only found in occasional cases.

In line with our observations, there was no association between CAP and parainfluenza viruses, enteroviruses, rhinoviruses or coronaviruses, and a negative association between CAP and human bocavirus in 361 children aged less than 5 years in a recent case-control study from Sweden [26]. In a recent study from the USA, rhinovirus was detected in 22 % of 2222 children with radiological CAP and after adjustment for age, in 17 % of 726 controls, and all other viruses were detected in less than 3 % of controls [13]. In the most recent nested case-control study from South-Africa including 314 pediatric CAP cases, RSV, pertussis, influenza virus, human bocavirus, adenoviruses and parainfluenza viruses were associated with pneumonia, but rhinoviruses were not [34]. In a recent study from Creek, 37.7 % of the 932 respiratory samples obtained from 233 apparently healthy children aged 2 to 5 years were virus-positive by PCR [1]. Human bocavirus was the most common finding, followed by rhinoviruses, in line with our findings. Repeated respiratory samples from 362 American children suggested that only 5 % of rhinovirus infections were associated with prolonged shedding, and others were new infections [18].

In conclusion, RSV, metapneumovirus, influenza A virus and adenoviruses were identified in the respiratory samples of children with CAP significantly more often than in healthy control children. The incidences of parainfluenza viruses, enteroviruses, coronaviruses and human bocavirus were equal in both groups, but rhinoviruses were found more often in non-symptomatic controls than in CAP patients. Our findings suggest that the role of rhinoviruses as a causative agent of CAP in children has been over-estimated, probably due to the non-controlled designs of previous studies.

Authors' Contributions TS and LK were responsible for the study plan and interpretation of the results, TS as a clinician and LK as a microbiologist. SY was responsible for PCR diagnostics for viruses and atypical infections. SK examined the patients and collected the data, MK participated in the interpretation and writing of the results.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The local Ethics Committee and the head doctor of the Children's City Polyclinic Number138, Moscow, Russia, approved the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from the parents or guardians of the children with CAP and oral informed consent was obtained from the parents or guardians of the control children.

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