Official Journal of the Asian Pacific Society of Respirology
Respirology

REVIEW

Incidence of viral infection detected by PCR and real-time PCR in childhood community-acquired pneumonia: A meta-analysis

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ABSTRACT

Several studies examining the incidence of viral infection in childhood community-acquired pneumonia (CAP) utilizing polymerase chain reaction (PCR) or real-time PCR methods have been reported. We systematically searched Pubmed and Embase for studies reporting the incidence of respiratory viral infection in childhood CAP. The pooled incidences of viral infection were calculated with a random-effects model. Sources of heterogeneity were explored by subgroup analysis and a univariant metaregression analysis. We included 21 eligible reports in our study. We found significant heterogeneity on the incidence of viral infection in childhood CAP. The random effects pooled incidence was 57.4% (95% confidence interval (CI): 50.8–64.1). The pooled incidence of mixed infection was 29.3% (95%CI: 23.0–35.6) with considerable heterogeneity. The pooled incidence of mixed infection was 29.3% (95%CI: 23.0–35.6). Rhinovirus, respiratory syncytial virus (RSV) and bocavirus were found to be the three most common viruses in childhood CAP. We also demonstrated that respiratory viruses were detected in 76.1% of patients aged ≤1 year, 63.1% of patients aged 2–5 years and 27.9% of patients aged ≥ 6 years. We conclude that respiratory viruses are widely detected in paediatric patients with CAP by PCR or real-time PCR methods. More than half of viral infections are probably concurrent with bacterial infections. Rhinovirus, RSV and bocavirus are the three most frequent viruses identified in childhood CAP; the incidence of viral infection decreased with age.

Key words: child, community-acquired pneumonia, incidence, meta-analysis, respiratory virus.

Abbreviations: CAP, community-acquired pneumonia; CI, confidence interval; PCR, polymerase chain reaction; RSV, respiratory syncytial virus.

INTRODUCTION

Childhood community-acquired pneumonia (CAP) as a common and serious health-care problem is responsible for one fifth of children's deaths according to the estimates of the World Health Organization.[1,2] Despite the development of antimicrobial agents and vaccines, the morbidity and mortality caused by childhood pneumonia remains substantial in both developing and developed countries.[3,4]

The establishment of the aetiological agents is essential for treatment decisions especially when the first-line antibiotics are ineffective. The contributions of bacterial agents to childhood CAP have been widely investigated. The burden of disease caused by respiratory viruses has probably been underestimated due to the poor sensitivity and specificity of conventional diagnostic methods for respiratory viruses.[5] However, recent advances in the molecular diagnostic techniques have improved the identification of respiratory viruses.[6] Several studies examining the incidence of viral infection in childhood CAP with polymerase chain reaction (PCR) or real-time PCR methods have been reported. However, systematic review and meta-analysis of those studies are lacking to establish the incidence of viral infection in childhood CAP.

We performed a meta-analysis to determine the incidence of viral infection detected by PCR or real-time PCR methods in paediatric patients with CAP and to report the incidence of different respiratory virus.

METHODS

Search strategy and study selection

We searched Pubmed and Embase for citations published before 31 August 2014 with free-word, keyword and MeSH retrieval as follows: 'community-acquired pneumonia', 'virus', 'pediatric', 'children', 'childhood', 'PCR', and 'polymerase chain reaction', 'real-time PCR'. Two authors independently screened titles and abstracts and retrieved the full text of any that appeared relevant. For inclusion, studies had to meet the following criteria[1]: being a cross-sectional, case–control or cohort study[2]; participants being ≤19 years

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Received 8 October 2014; invited to revise 24 November 2014; revised 30 November 2014; accepted 4 December 2014 (Associate Editor: Marcos Restrepo).

Article first published online: 23 January 2015

old[3]; either reporting viral incidence or providing raw data to enable their calculation[4]; detecting respiratory viruses with PCR or real-time PCR methods[5]; and full text available in English or Chinese literature. We excluded studies in adult or those using conventional methods for viral detection. We also excluded studies that evaluated the incidence of one specific respiratory virus.

Data extraction and classification

All included studies were quality independently assessed by two authors using quality criteria (Supplementary Table S1) based on the standard principles from Strengthening the Reporting of Observational studies in Epidemiology.[7] For each study, one author extracted the information as follows: author name, country, year of publication, participants (number and mean age), specimens, viral detection methods and outcomes (the number of overall viral infection, the number of viral infection mixed with other pathogens and the number of individual viral infection); a second author checked for accuracy.

Statistical analysis

We used DerSimonian–Laird random-effects meta-analysis[8] to calculate the pooled incidence of viral infection (with 95% confidence intervals (CI)) because of anticipated heterogeneity across studies. We tested for heterogeneity across the studies with Cochran Q (heterogeneity χ^2) and I^2 statistic (30–60% for moderate heterogeneity; 50–90% for substantial heterogeneity; 75–100% for considerable heterogeneity).[9] We performed subgroup analysis in order to reduce the heterogeneity across studies and conduct further analysis. We also explored potential sources of heterogeneity by applying a univariate metaregression analysis examining: geographical region, specimen, the number of virus detected and detection methods.[10] We assessed publication bias in our meta-analyses with the Egger tests and Begg–Mazumdar tests. We performed all analyses in Stata 12.1 (StataCorp, College Station, TX, USA) with the commands metan (for random-effects meta-analysis) and metareg (for metaregression).

RESULTS

Our searches returned a total of 337 records, out of which 46 were excluded as duplicates. After assessing all citations by titles and abstracts, we reviewed 55 papers in full. After exclusion of ineligible reports, 21 studies reporting on 10 196 participants (n = 10196) published between April 2000 and August 2014 were included in our analysis[11–31] (Fig. 1). Quality scores were reported in Table 1.

Of these 21 reports, eight studies enrolled participants aged ≤5 years,[16,17,19,20,24,26,27,29] and the other 13 studies enrolled participants aged ≤19 years.[11–15,18,21–23,25,28,30,31] Twelve were carried out in Europe,[12,14,15,18,20–22,26,28–31] five in Asia[13,16,23,25,27] and four in other regions (one in the USA,[11] one in Mozambique,[17] one in Brazil[24] and one in Israel[19]). Eleven studies detected respiratory viruses solely based on PCR or real-time PCR[11–13,15–17,20,23,25,28,31] while the other 10 studies applied PCR or real-time PCR techniques combined with conventional methods for virus detection.[14,18,19,21,22,24,26,27,29,30]

Overall incidence of respiratory viral infection in childhood CAP ranged from 18.7% to 91.0%

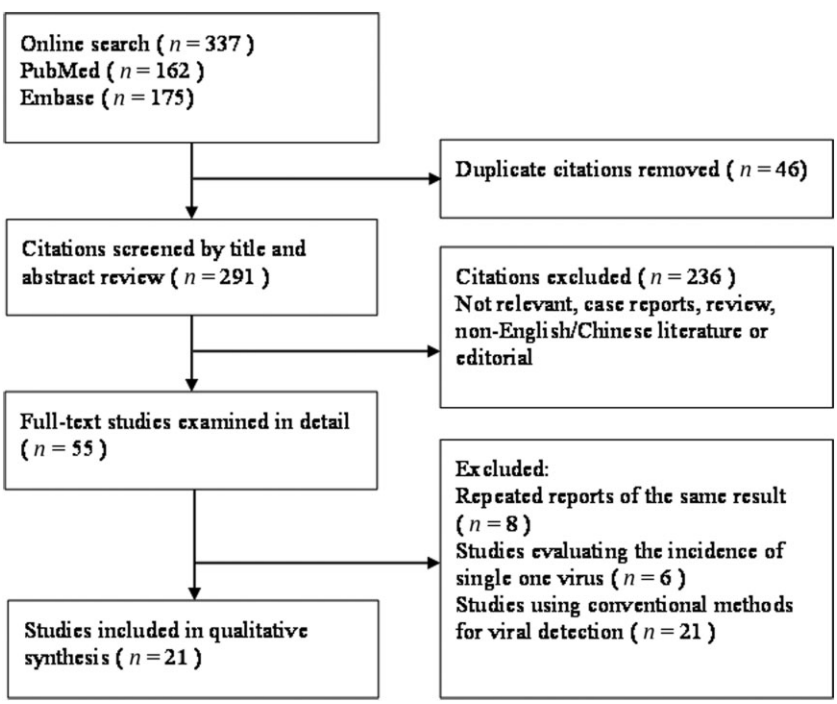

Figure 1 Flow diagram of the study selection process.

Table 1 Summarized information and quality assessment of the studies included in meta-analysis

Study	Year of publication	Country	Patients	Specimens	Methods	Number of viruses detected	Quality score[†]
Cantais *et al.*[31]	2014	France	<16 years	Induced sputum	Real-time PCR	15	6
Wiemken *et al.*[11]	2013	USA	<18 years	Nasopharyngeal swabs	PCR	12	5
Esposito *et al.*[12]	2013	Italy	<14 years	Respiratory secretion samples	Real-time PCR	17	9
Okada *et al.*[13]	2012	Japan	<15 years	Nasopharyngeal swabs	Real-time PCR	11	8
Honkinen *et al.*[14]	2012	Finland	<15 years	Induced sputum samples	Fluoroimmunoassay, real-time PCR	18	7
Ding *et al.*[16]	2012	China	<5 years	Nasopharyngeal aspirates	Real-time PCR	12	6
Garcia-Garcia *et al.*[15]	2012	Spain	<14 years	Nasopharyngeal aspirates	PCR	16	7
De Schutter *et al.*[18]	2011	Belgium	<14 years	BALF	Culture, PCR	10	5
O'Callaghan-Gordo *et al.*[17]	2011	Mozambique	<5 years	Nasopharyngeal aspirate	PCR	12	7
Wolf *et al.*[19]	2010	Israel	<5 years	Nasopharyngeal wash specimens	DFA, PCR	8	5
Mathisen *et al.*[20]	2009	Norway	<3 years	Nasopharyngeal aspirate	PCR	7	6
Lahti *et al.*[21]	2009	Finland	6 months to 15 years	Nasopharyngeal aspirate and induced sputum	Fluoroimmunoassay, real-time PCR	11	6
Cevey-Macherel *et al.*[22]	2009	Switzerland	2 months to 5 years	Serum, nasopharyngeal aspirates	Serology, DFA, real-time PCR	13	7
Samransamruajkit *et al.*[23]	2008	Thailand	1 month to 15 years	Nasopharyngeal samples	Real-time PCR	7	6
Nascimento-Carvalho *et al.*[24]	2008	Brazil	<5 years	Serum, nasopharyngeal aspirates	Serology, DFA, PCR	8	5
Hamano-Hasegawa *et al.*[25]	2008	Japan	<18 years	Nasopharyngeal samples	Real-time PCR	13	7
Cilla *et al.*[26]	2008	Spain	<3 years	Nasopharyngeal samples	Culture, PCR	14	6
Nakayama *et al.*[27]	2007	Japan	<5 years	Serum,nasopharyngeal samples	Serology, PCR	11	7
Tsolia *et al.*[28]	2004	Greece	<14 years	nasopharyngeal wash samples	PCR	10	7
Laundy *et al.*[29]	2003	UK	<5 years	Nasopharyngeal aspirate	PCR, IFA	8	6
Juven *et al.*[30]	2000	Finland	<14 years	Nasopharyngeal sample	Culture, IFA, PCR	12	6

[†] Maximum score = 9.

BALF, bronchial alveolar lavage fluid; DFA, direct immunofluorescence assay; IFA, indirect immunofluorescence assay; PCR, polymerase chain reaction.

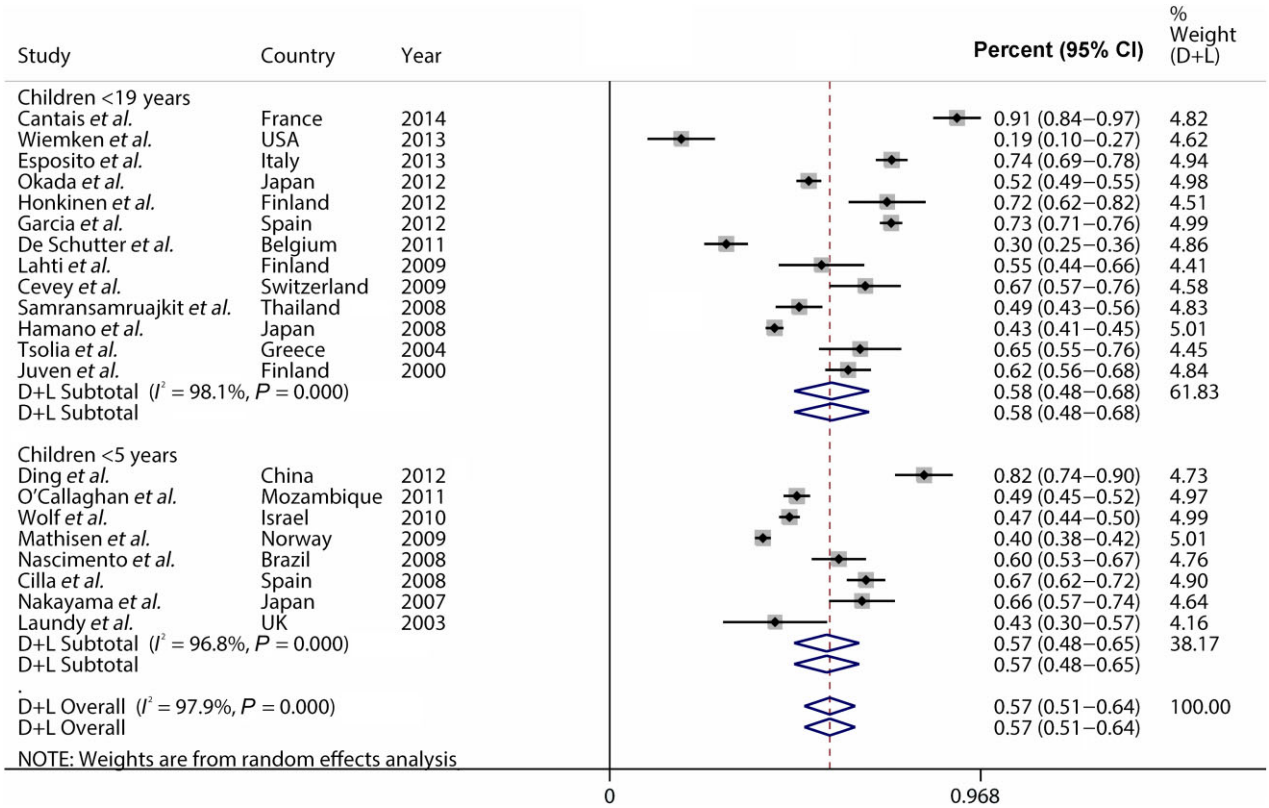

Figure 2 The pooled incidence of viral infection in childhood community-acquired pneumonia (CAP).

Table 2 The pooled incidence of viral infection and mixed infection in paediatric patients with CAP stratified by regions where studies were performed

	Incidence (%)	95% CI	χ^2	*P*	I^2 (%)
The pooled incidence of viral infection					
Europe	61.7	50.–70.3	561.99	0.000	98.2
Asia	58.0	47.1–68.8	116.58	0.000	96.6
Other regions	44.2	34.6–53.8	53.22	0.000	94.4
The pooled incidence of mixed infection					
Europe	33.8	18.0–49.5	186.46	0.000	97.3
Asia	23.7	15.8–31.5	38.77	0.000	94.8
Other regions	29.3	23.0–35.6	23.7	0.000	95.8

CAP, community-acquired pneumonia; CI, confidence interval.

(Fig. 2); heterogeneity was considerable (χ^2 = 781.4, P < 0.0001; I^2 = 97.9%). The random effects pooled incidence was 57.4% (95% CI: 50.8–64.1). Due to the significant heterogeneity, pooled incidence of viral infection was calculated stratified by participants (≤5 years old or ≤19 years old, as illustrated in Fig. 2) or by geographical region where each study was carried out (Europe, Asia and other regions) as shown in Table 2. The pooled incidence of overall respiratory viral infection was 56.6% (95% CI: 48.1–65.1, I^2 = 96.8%) in participants ≤5 years old and 57.9% (95% CI: 48.1–67.7, I^2 = 98.1%) in participants ≤19 years old. In the subgroup analysis according to geographical region, the pooled incidence in Europe was similar to that in Asia (59.1%, 95% CI: 47.8–70.3, I^2 = 98.2%; 58.0%, 95% CI: 47.1–68.8). However, considerable heterogeneity persisted in subgroup analysis. In individual variable metaregression analysis, high number of virus for detection was related to high incidence of viral detection (Table 3).

Among the 21 reports, 11 studies provided raw data to estimate incidence of viral infections mixed with other pathogens (n = 4169).[12–14,18,21,22,24,25,27,28,30,31] Incidence estimates of mixed infections ranged from 6.4% to 66.0%; heterogeneity was considerable (χ^2 = 230.3, P < 0.0001; I^2 = 96.1%). The pooled incidence of mixed infection was 29.3% (95% CI: 22.4–36.2, I^2 = 96.1%). Similarly as above, subgroup analysis was carried out

Table 3 Univariate metaregression for incidence of viral infections and mixed infection in pediatric patients with CAP

	Metaregression coefficient	95% CI	*P*
Incidence of respiratory viral infection in childhood CAP			
Specimen	0.139	−0.028 to 0.307	0.098
Region	−0.082	−0.177 to 0.013	0.087
The number of virus detected	0.034	0.013 to 0.054	0.003
Viral detection methods	0.003	−0.091 to 0.096	0.953
Incidence of respiratory viral infection mixed with other pathogens in childhood CAP			
Specimen	0.064	−0.141 to 0.269	0.503
Region	−0.109	−0. 219 to 0.001	0.052
The number of virus detected	0.033	−0. 001 to 0.068	0.049
Viral detection methods	0.113	0. 032 to 0.194	0.011

CAP, community-acquired pneumonia; CI, confidence interval.

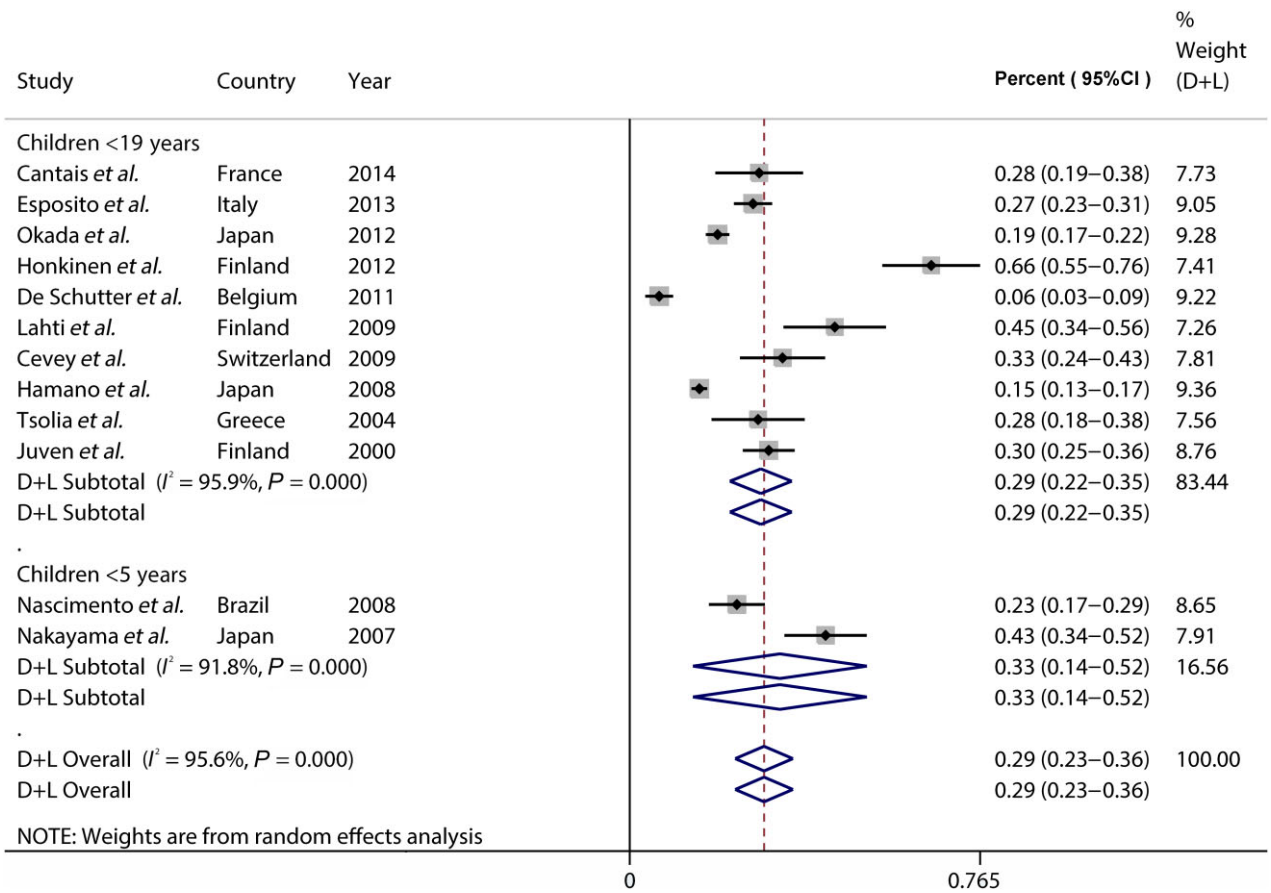

Figure 3 The pooled incidence of viral infection mixed with other pathogens in childhood community-acquired pneumonia (CAP).

according to participants (≤5 years old or ≤19 years old) or the regions. The pooled incidence of mixed infections was 32.8% (95% CI: 13.8–51.7, I^2 = 91.8%) in patients ≤5 years old, 29.4% (95% CI: 21.8–35.2, I^2 = 96.4%) in patients ≤19 years old. Results of subgroup analysis according to regions were demonstrated in Table 2, along with considerable heterogeneity.

We further estimated individual incidence of common respiratory virus. As shown in Figure 3 and Table 4, the pooled incidence of childhood CAP associated with rhinovirus was highest (18.9%, 95% CI: 14.3–23.4, I^2 = 95.8%), followed by respiratory syncytial virus (RSV) (17.5%, 95% CI: 13.3–21.6, I^2 = 97.1%) and bocavirus (12.7%, 95% CI: 8.5–16.9, I^2 = 95.8%). The incidence of virus detected was higher in studies when real-time PCR was used for virus detection compared with other detection methods (Table 3).

Several studies provided incidence of viral infection in patients stratified by age: ≤1 year, 2–5 years, or ≥6

Table 4 Discrepancies of the pooled incidence among the common respiratory viruses

	Incidence (%)	95% CI	χ^2	*P*	I^2 (%)
RSV	17.5	13.3–21.6	621.22	0.000	97.1
Rhinovirus	18.9	14.3–23.4	306.04	0.012	95.8
Influenza	6.3	4.7–8.0	242.90	0.000	92.6
hMPV	6.1	4.1–8.1	379.59	0.003	96.0
Bocavirus	12.7	8.5–16.9	167.50	0.000	95.8
Parainfluenza	7.8	6.0–9.5	211.04	0.001	91.5
Adenovirus	6.0	4.4–7.7	212.01	0.019	92.5
Coronavirus	3.9	2.1–5.7	40.65	0.000	82.8

CI, confidence interval; RSV, respiratory syncytial virus; hMPV, human metapneumovirus.

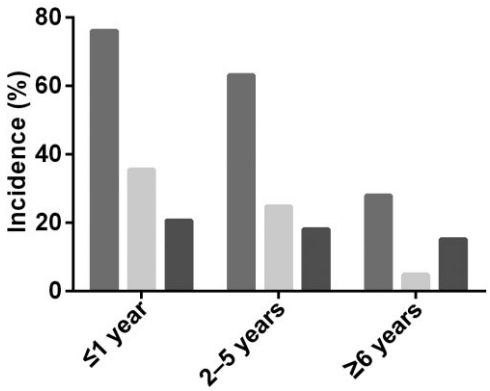

Figure 4 The pooled incidence of childhood community-acquired pneumonia (CAP) associated with respiratory viruses, rhinovirus or respiratory syncytial virus (RSV) stratified by age. (■) Overall; (■) RSV; (■) rhinovirus.

years old.[12,13,24,26,27,30] The pooled incidence of viral infection was 76.1% (95% CI: 62.8–89.4, I^2 = 95.1%) in patients aged ≤1 year, 63.1% (95% CI: 50.2–75.9, I^2 = 94.1%) in patients aged 2–5 years and 27.9% (95% CI: 4.3–51.5, I^2 = 96.3%) in patients aged ≥6 years old. Our study indicated that the incidence of RSV-positive CAP in children varied with age as shown in Figure 4. The pooled incidence of RSV-positive CAP was 35.5% (95% CI: 22.0–49.0, I^2 = 90.2%) in patients aged ≤1 year, 24.8% (95% CI: 14.3–35.3, I^2 = 92.6%) in patients aged 2–6 years and 4.8% (95% CI: 0.0–11.3, I^2 = 86.6%) in patients aged ≥6 years old. The incidence of rhinovirus infections was similar in the three age groups.

We estimated publication bias with Egger tests and Begg–Mazumdar tests. However, no publication bias was identified (Supplementary Fig. S1).

DISCUSSION

Our systematic review and meta-analysis included 21 previously published reports investigating the incidence of viral infection in childhood CAP. Our main findings are that respiratory viruses could be detected in approximately 55% paediatric patients with CAP, with more than half characterized as mixed infection. Rhinovirus, RSV and bocavirus were the most frequently detected pathogens in childhood CAP. The incidence of viral infection varied with age and in particular was higher in patients aged ≤1 year old than that in patients aged ≥6 years old. The findings elucidate the contributions of respiratory virus in causing childhood CAP.

Rhinovirus, RSV and bocavirus were the three most common viruses associated with childhood CAP, while influenza virus, rhinovirus and coronavirus are the leading viruses in adult patients with CAP.[32,33] Contrary to RSV, which has been clearly defined as an important cause of childhood CAP, rhinoviruses and bocavirus were uncommon findings using conventional methods such as culture, antigen detection or serology. However, with the advent of PCR techniques, rhinoviruses and bocavirus have been detected increasingly in childhood CAP.[34,35] Our findings emphasize the importance of these viruses which are involved in the pathogenesis of childhood CAP and underline the need to address this clinical problem. Up to now, experience with antivirals for CAP caused by these viruses is scarce. Only few case reports and some treatment studies in immunosuppressed patients investigated the efficiency of ribavirin, which is a broad antiviral agent in treatment for bronchiolitis and pneumonia caused by RSV infection.[36,37] More safe and efficient vaccines and agents are needed to be developed in order to prevent and manage these viral infections.

As demonstrated in our study, mixed infection by viruses and other pathogens account for more than half of overall viral infection. Interaction of virus and bacteria in the pathogenesis of pneumonia has been partially explored. One hypothesis is that viral infections are followed by secondary bacterial infection.[38,39] Viral infections disrupt mucosal barriers in the respiratory tracts, which makes hosts susceptible to bacterial infection. Mixed infections may induce more severe clinical diseases than individual bacterial or viral infections alone. One study reported that co-infection of influenza virus and *Staphylococcus aureus* can lead to severe fatal pneumonia in children.[40,41]

Our heterogeneity analysis generated two key findings. Firstly, the incidence of overall virus infection is reported to be higher in the studies that detect many virus species than the studies which detect fewer

species. The yield virus detection is associated with the species viruses identified. Secondly, for mixed infection, real-time PCR achieve higher yield rate compared with other diagnostic methods. This result highlights the importance to develop standards for identifying respiratory virus in clinical practice.

Our study has several limitations. First of all, only reports in English/Chinese literature were included in our study, which led to the loss of raw data from reports in other languages. Furthermore, many studies indicated that some respiratory viruses present a strong seasonal pattern like influenza viruses. However, those data were not included for the meta-analysis. Moreover, we did not correlate clinical severity of pneumonia with causative viral pathogens due to the lack of original data.

In conclusion, our results suggest that more attention should be paid to the respiratory viruses as a cause or contributing factor of childhood CAP. Further studies are required to establish a standard method for specimen collection and identification of respiratory viruses.

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Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's web-site:

Supplementary Figure S1 Estimation of publication bias with Egger tests.

Supplementary Table S1 Quality assessment.

