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 univariate 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.

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: being a cross-sectional, case–control or cohort study; participants being ≤19 years.
old; either reporting viral incidence or providing raw data to enable their calculation; detecting respiratory viruses with PCR or real-time PCR methods; and full text available in English or Chinese literature. We excluded studies in adults 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. 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 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 $\chi^2$) and $I^2$ statistic (30–60% for moderate heterogeneity; 50–90% for substantial heterogeneity; 75–100% for considerable heterogeneity). 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. 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 (Fig. 1). Quality scores were reported in Table 1.

Of these 21 reports, eight studies enrolled participants aged ≤5 years, and the other 13 studies enrolled participants aged ≤19 years. Twelve were carried out in Europe, five in Asia and four in other regions (one in the USA, one in Mozambique, one in Brazil and one in Israel). Eleven studies detected respiratory viruses solely based on PCR or real-time PCR, while the other 10 studies applied PCR or real-time PCR techniques combined with conventional methods for virus detection.

Overall incidence of respiratory viral infection in childhood CAP ranged from 18.7% to 91.0%
| Study               | Year of publication | Country    | Patients | Specimens                  | Methods                           | Number of viruses detected | Quality score† |
|---------------------|---------------------|------------|----------|----------------------------|----------------------------------|-----------------------------|----------------|
| Cantais et al.      | 2014                | France     | <16 years| Induced sputum             | Real-time PCR                    | 15                          | 6              |
| Wiemken et al.      | 2013                | USA        | <18 years| Nasopharyngeal swabs       | PCR                              | 12                          | 5              |
| Esposito et al.     | 2013                | Italy      | <14 years| Respiratory secretion samples| Real-time PCR                    | 17                          | 9              |
| Okada et al.        | 2012                | Japan      | <15 years| Nasopharyngeal swabs       | Real-time PCR                    | 11                          | 8              |
| Honkinen et al.     | 2012                | Finland    | <15 years| Induced sputum samples     | Fluoroimmunoassay, real-time PCR | 18                          | 7              |
| Ding et al.         | 2012                | China      | <5 years | Nasopharyngeal aspirates   | Real-time PCR                    | 12                          | 6              |
| Garcia-Garcia et al.| 2012                | Spain      | <14 years| Nasopharyngeal aspirates   | PCR                              | 16                          | 7              |
| De Schutter et al.  | 2011                | Belgium    | <14 years| BALF                        | PCR                              | 10                          | 5              |
| O’Callaghan-Gordo et al. | 2011      | Mozambique | <5 years | Nasopharyngeal aspirate        | PCR                              | 12                          | 7              |
| Wolf et al.         | 2010                | Israel     | <5 years | Nasopharyngeal wash specimens | DFA, PCR                         | 8                           | 5              |
| Mathisen et al.     | 2009                | Norway     | <3 years | Nasopharyngeal aspirate     | PCR                              | 7                           | 6              |
| Lahti et al.        | 2009                | Finland    | 6 months to 15 years | Nasopharyngeal aspirate and induced sputum | Fluoroimmunoassay, real-time PCR | 11                          | 6              |
| Cevey-Macherel et al.| 2009               | Switzerland | 2 months to 5 years | Serum, nasopharyngeal aspirates | Serology, DFA, real-time PCR | 13                          | 7              |
| Samransamruajkit et al. | 2008     | Thailand   | 1 month to 15 years | Nasopharyngeal samples          | Real-time PCR                     | 7                           | 6              |
| Nascimento-Carvalho et al. | 2008 | Brazil    | <5 years | Serum, nasopharyngeal aspirates | Serology, DFA, PCR | 8                           | 5              |
| Hamano-Hasegawa et al. | 2008     | Japan      | <18 years | Nasopharyngeal samples      | Real-time PCR                     | 13                          | 7              |
| Cilla et al.        | 2008                | Spain      | <3 years | Nasopharyngeal samples      | Culture, PCR                      | 14                          | 6              |
| Nakayama et al.     | 2007                | Japan      | <5 years | Serum, nasopharyngeal samples | Serology, PCR                     | 11                          | 7              |
| Tsolia et al.       | 2004                | Greece     | <14 years| Nasopharyngeal wash samples | PCR                              | 10                          | 7              |
| Laundy et al.       | 2003                | UK         | <5 years | Nasopharyngeal aspirate     | PCR, IFA                         | 8                           | 6              |
| Juven et al.        | 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.
heterogeneity was considerable ($\chi^2 = 781.4$, $P < 0.0001; I^2 = 97.9\%$). 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, $P = 96.8\%$) in participants ≤5 years old and 57.9% (95% CI: 48.1–67.7, $P = 98.1\%$) 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, $P = 98.2\%$; 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 ($n = 4169$). The random effects pooled incidence of mixed infection was 29.3% (95% CI: 22.4–36.2, $P = 96.1\%$). Similarly as above, subgroup analysis was carried out (Fig. 2); heterogeneity was considerable ($\chi^2 = 230.3$, $P < 0.0001; I^2 = 96.1\%$).
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, \( I = 95.8\% \)), followed by respiratory syncytial virus (RSV) (17.5%, 95% CI: 13.3–21.6, \( I = 97.1\% \)) and bocavirus (12.7%, 95% CI: 8.5–16.9, \( I = 95.8\% \)). 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 3

| Specimen | Region | The number of virus detected | Viral detection methods |
|----------|--------|-----------------------------|-------------------------|
| Incidence of respiratory viral infection in childhood CAP | 0.139 | −0.028 to 0.307 | 0.098 |
| | −0.082 | −0.177 to 0.013 | 0.087 |
| | 0.034 | 0.013 to 0.054 | 0.003 |
| | 0.003 | −0.091 to 0.096 | 0.953 |

| Specimen | Region | The number of virus detected | Viral detection methods |
|----------|--------|-----------------------------|-------------------------|
| Incidence of respiratory viral infection mixed with other pathogens in childhood CAP | 0.064 | −0.141 to 0.269 | 0.503 |
| | −0.109 | −0.219 to 0.001 | 0.052 |
| | 0.033 | −0.001 to 0.068 | 0.049 |
| | 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).
years old. The pooled incidence of viral infection was 76.1% (95% CI: 62.8–89.4, $I^2 = 95.1\%$) in patients aged $\leq 1$ 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 $\geq 6$ 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 $\leq 1$ 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 $\geq 6$ 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 $\leq 1$ year old than that in patients aged $\geq 6$ 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. 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. 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. 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. 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.

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.