Etiology and Clinical characteristics of bronchiolitis in Suzhou

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Abstract

Background

Bronchiolitis is a clinical syndrome commonly encountered in practice, particularly among infants and young children. To investigate the prevalence of pathogens in hospitalized infants with bronchiolitis and study the relationship between the clinical characteristics and pathogens.

Methods

We investigated the respiratory specimens and clinical data of 1012 children with bronchiolitis who were treated at the Children’s Hospital of Soochow University between November 2011 and December 2018. The nasopharyngeal aspirates were examined by direct immunofluorescence assay or polymerase chain reaction (PCR) to detect viruses and by PCR and enzyme-linked immunosorbent assay to detect Mycoplasma pneumoniae (MP).

Results

Of the 1012 children with bronchiolitis, 842 (83.2%) were detected at least a pathogen. 614 (60.7%) had single viral infections, 91 (9.0%) had MP infections, 70 (6.9%) had multiple viral infections, and 67 (6.6%) had mixed viral and MP infection. The most common pathogens detected were respiratory syncytial virus (RSV) (44.4%), MP (15.6%), and human rhinovirus (HRV) (14.4%). RSV was the most common pathogen detected in children less than 6 months. Coinfection was detected in 13.5% (137/1012) of the children, but it was less common in children less than 6 months. The age of children with single virus infection was the youngest. Children with single virus infection had a higher proportion of oxygen therapy compared with single MP infection.

Conclusions

The most common pathogen detected in children with bronchiolitis is RSV, followed by MP and HRV. Co-infections lead to prolonged illness and worsening of the symptoms

Keywords: Etiology, Bronchiolitis, Infant, Respiratory syncytial virus, Mycoplasma pneumoniae

Background

Bronchiolitis is an acute infection of the lower respiratory tract, particularly affecting the terminal and respiratory bronchioles, with the possibility of extending to the adjacent alveolar ducts and spaces.
Bronchiolitis is the most frequent disease in children < 2 years and is the leading cause of hospital admissions in this age group. Bronchiolitis is a well-known clinical entity, which affects around 1%-3% of all healthy children,² and the mortality rate reported for this condition is reported to be approximately 2 per 100000 infants.³ Currently, there is no effective curative treatment for bronchiolitis. However, the mortality rate of bronchiolitis has even been reported to be 3.5% in China,⁴ which we should pay more attention on.

Respiratory syncytial virus (RSV) is the most common viral pathogen identified in children with globally, acute lower respiratory infection (ALRI); about 45% of hospital admissions and in-hospital deaths due to RSV-ALRI occur in children younger than 6 months.⁵ Also, RSV is the most common pathogen identified in bronchiolitis, followed by parainfluenza virus and adenovirus.⁶,⁷ Furthermore, recent studies have determined that bacterial pathogens, particularly *Mycoplasma pneumoniae* (MP) and *Chlamydophila pneumoniae* (CP), are responsible for bronchiolitis in children under 2 years of age.⁸–¹⁰ However, the clinical relevance of the various pathogens involved in children still remains unclear.

In this study, we sought to evaluate the distribution of pathogens responsible for bronchiolitis in children ≤ 2 years of age and analyze the differences in the clinical features of bronchiolitis caused by different pathogenic agents.

**Methods**

**Subjects**

We conducted a retrospective analysis of the data of 1012 children who were admitted to the Children’s Hospital of Soochow University for the management of bronchiolitis between November 2011 and December 2018. Children’s Hospital of Soochow University is a tertiary referral center at Jiangsu Province, East China. It has over 1000 beds and 50,000 inpatients annually. The inclusion criteria for this study were children aged between 1 month and 2 years, occurrence of first episode of wheezing, and clinical evidence of bronchiolitis (tachypnoea, wheeze, prolonged expiratory phase, and crackles on auscultation). This study protocol was approved by the Medical Ethics Committee of
Specimen Collection

Within 24 hours of admission, nasopharyngeal aspiration was performed to collect specimens from all patients. For aspiration, a suction catheter was used introduced through the nose and advanced into the lower portion of the pharynx, up to a distance of 7-9 cm. Nasopharyngeal aspirate of 2 mL was then collected and sent for histopathological analysis within 30 min of collection. The retrieved sample was centrifuged (500×g, 10 min) and suspended in 2 mL saline and separated into 2 aliquots for direct immunofluorescence assay (DFA) and polymerase chain reaction (PCR) to identify pathogens.

Microbe Detection

A quantitative diagnostic kit (provided by Sun Yat-sen University Daan Gene Co., Ltd.) for MP DNA was performed to identify the 16s rRNA gene of MP extracted from nasopharyngeal specimens.\textsuperscript{11} DFA was performed to detect RSV, influenza virus (IV), parainfluenza virus (PIV), and adenovirus (ADV). The assay kits were obtained from Chemicon (USA), and all staining procedures were performed according to the manufacturer’s instructions. Immunofluorescence studies were then conducted (Leica 020-518.500, Germany). RNA was extracted from the specimens using Trizol reagent (Invitrogen, USA), followed by cDNA synthesis by reverse transcription. The cyclic temperature settings were 94°C for 30s; 55°C for 30s; followed by 68°C for 30s; and, after 45 amplification cycles, a final extension was performed at 68°C for 7 min. For detection of human metapneumovirus (hMPV) and rhinoviruses (HRV) fluorescent real-time PCR (BIO-RAD iCycler) was performed. DNA extraction and real-time PCR were to detect the human bocavirus (HBoV).

Data Collection

The medical records of the patients were reviewed and data regarding the following parameters were recorded: (1) demographic and clinical characteristics, including age, gender, and duration of symptoms prior to admission; (2) results of viral diagnostic tests performed in nasopharyngeal aspirates; (3) results of blood tests for inflammatory indices, including white blood cell (WBC) count, percentage of neutrophils, serum C-reactive protein (CRP) levels.
**Statistical Analysis**

All statistical analyses in this study were performed using the Statistical Package for the Social Sciences (version 25.0). Frequency distributions and rates were used for descriptive analyses. Parameters regarding the patient’s demographic data and baseline characteristics were analyzed using means (SD) or medians (25th-75th percentiles). Normal distribution was met by the data, as confirmed by using t-test variance analysis. For parameters without the non-normal distribution of data, the Wilcoxon rank sum test and Kruskal-Wallis H test was used for comparison between two groups and multiple groups, respectively. \(P\) value of < 0.05 was considered to indicate statistical significance.

**Results**

**Patients**

Among the 1012 children with bronchiolitis enrolled in this study, at least one pathogen was detected in 842 (83.2%) children. Among these patients, 603 (71.6%) were male and 239 (28.4%) were female, with ages ranging from 1 month to 24 months (median: 5 months). The median duration of symptoms before admission was 6 days. With respect to clinical presentation, 372 (44.2%) had sneezing, 241 (28.6%) had fever, 172 (20.4%) had tachypnea, 51 (6.1%) had dyspnea, and 164 (19.5%) required oxygen administration.

**Etiology**

The most common pathogens detected were RSV (44.4%), MP (15.6%), HRV (14.4%), human bocavirus (HBoV; 9.8%), and PIV (8.0%). Co-infection was identified in 137 (13.5%) of the patients. Further, among the patients, 62.3% were \(\leq\) 6 months old; 24% were 6 months to \(\leq\) 1 years old and 13.7% of patients were 1 to 2 years old. The most common pathogens isolated were RSV (58.9%), HRV (11.6%), MP (11.3%), and PIV (8.8%) in patients aged \(\leq\) 6 months. On other hand, in the patients of ages between 6 months and \(\leq\) 1 years, the most common pathogens were RSV (27.7%), MP (22.1%), HBoV (18.6%), and HRV (17.8%). For 1 to 2-year-old children, the most common pathogens were MP (24.3%), HRV (22.2%), RSV (20.1%), and HBoV (18.1%). Thus, for children under 6 months of age, RSV was identified as the most common pathogen responsible for bronchiolitis, while MP
infection was less common (all \( P < 0.002 \)) (Figure 1).

**Mixed infections**

Of the 842 patients, 614 (72.9%) had single viral infections; 91 (10.8%) had only MP infections; 70 (8.3%) had multiple viral infections, and 67 (8.0%) had viral infection mixed with MP. Among patients aged \( \leq 6 \) months, 12% had co-infection, with 6.5% having multiple viral infections and 5.5% having coinfection of a virus and MP. Among patients between 6 months and \( \leq 1 \) years of age, 22.8% had co-infection, with 11.4% having mixed viral-viral infections and 11.4% having viral-MP infections. In the patients aged 1 to 2 years, 24.3% had co-infection, with 11.3% and 14.0% having mixed viral-viral and viral-MP co-infections, respectively. The probability of co-infection in children of age \( \leq 6 \) months was significantly lower than in children of age between 6 months and \( \leq 2 \) years (all \( P < 0.002 \)).

| Pathogens Identified in Hospitalized Children with Bronchiolitis |
|---------------------------------------------------------------|
| **Pathogen** | **No. of Episodes** | **Total No. of Episodes** |
|               | Single Infection | Coinfection With Viruses | Coinfection With MP |
| RSV           | 377 (84.0)       | 43 (9.6)                  | 29 (6.4)             | 449 |
| HRV           | 86 (58.9)        | 43 (29.5)                 | 17 (11.6)            | 146 |
| HBoV          | 58 (58.6)        | 29 (29.3)                 | 12 (12.1)            | 99  |
| PIV           | 61 (75.3)        | 10 (12.3)                 | 10 (12.3)            | 81  |
| IV            | 16 (45.7)        | 15 (42.9)                 | 4 (11.4)             | 35  |
| hMPV          | 12 (75.0)        | 1 (6.3)                   | 3 (18.8)             | 16  |
| ADV           |                 | 4                         | 2                    | 7   |
| Atypical pathogen | 91 (57.6) | 67 (42.4) | — | 158 |

\( ^a \) Data are n (%).

\( ^b \) The percentages are not listed because the total episodes is too small.

**Comparisons of clinical characteristics of the patients with single and mix infections**

Comparison of the demographic and clinical features of the children shows that children with single virus infection was the youngest (mean age: 5.58 months). Further, the number of children presenting with fever and percentage of neutrophils were the lowest among children infected with a single virus (all \( P < 0.001 \)). The median duration of symptoms before admission of children with single MP infection (9 days), as well as those with viral-MP infection (7 days) was significantly greater than that of children infected with single virus (5 days) (\( P < 0.001 \), respectively). The mean length of stay of
children with single virus infection (8.1 days), as well as those with viral-MP infection (8.0 days) was significantly less than that of children infected with viral-MP (9.0 days) \((P < 0.05, \text{ respectively})\).

Children with single virus infection had a higher proportion of oxygen therapy compared with single MP infection \((21.7\% \text{ VS } 8.8\%, P=0.004)\), while the PICU admission rate didn’t differ between the two groups. (Table 2).

Table 2 The Demographic and Clinical Characteristics of 842 Patients with Bronchiolitis Associated with Single/mix infections

| Characteristics            | Single virus | Single MP | Mixed viruses | Mixed viruses/MP |
|----------------------------|--------------|-----------|---------------|------------------|
| No. of patients            | 614          | 91        | 70            | 67               |
| Age, months \(^a\)         | 5.58\(^{cde}\) | 8.80\(^c\) | 7.51\(^d\)   | 8.1              |
| Gender, \% male            | 72.5         | 67        | 74.3          | 67.              |
| Duration of symptoms before admission, days \(^b\) | 5\(^c,d\) | 9\(^c\) | 7             | 7\(^c\)          |
| Length of stay \(^a\)      | 8.1\(^c\)    | 8.0\(^d\) | 8.2           | 9.0              |
| Fever, \%                  | 23.6\(^{cde}\) | 40.7\(^c\) | 35.7\(^d\)   | 50.              |
| Nasal congestion, \%       | 45.8         | 31.9      | 41.4          | 49.              |
| Tachypnoea, \%             | 21.5         | 13.2      | 20            | 17.              |
| Dyspnea, \%                | 6.8          | 2.2       | 5.7           | 4.\(^!\)          |
| WBC count, \(\times 10^9/L\) \(^a\) | 9.77 | 10.20 | 9.72 | 10.\(^!\) |
| Percentage of neutrophils \(^a\) | 33.3\(^{cde}\) | 39.05\(^c\) | 38.46\(^d\) | 40.7           |
| CRP, mg/L\(^b\)            | 0.39         | 0.76      | 0.34          | 0.9              |
| Need of oxygen, \%          | 21.7\(^{cd}\) | 8.8\(^{ce}\) | 11.4\(^d\)   | 22.              |
| PICU admission, \%          | 10.6         | 5.5       | 10            | 7\(^!\)          |

\(^a\)The mean value was used.
\(^b\) The median value was used.
\(^c\) Significant differences were observed between each pair of values.
\(^d\) Significant differences were observed between each pair of values.
\(^e\) Significant differences were observed between each pair of values.

Discussion

Bronchiolitis is the most frequent disease affecting children of age < 2 years, and it is a leading cause of hospital admissions in this age group. In this study, we conducted a retrospective investigation of 842 children hospitalized with bronchiolitis in order to identify the distribution of pathogens and co-infection. We found that 83.7\% of the children had a single pathogen infection, whereas 16.3\% had co-infections. The sex ratio of male and female was 2.52:1. Males have short and narrow airways may be more likely to occur bronchiolitis,\(^12\) and males are approximately twice as likely to become hospitalized than females due to RSV infection.\(^13\) A new retrospective study in Italy also shows that
there is a higher incidence of bronchiolitis in boys than in girls.\textsuperscript{14} Specific pathogenic mechanisms between RSV and gender need further exploration.

The most common pathogens identified in our study were RSV (53.5\%), followed by MP (18.8\%) and HRV (17.3\%). A longitudinal prospective investigation conducted in the USA revealed that RSV was the causative pathogen for bronchiolitis in 76\% of infants; this percentage is much higher than that observed in our study; however HRV was isolated in 18\% of the their cases, which is similar to the rate of HRV infection noted in our study.\textsuperscript{15} In an 11-year study in Spain, 62.7\% of the patients (children ≤ 2 years of age with acute bronchiolitis) had RSV infections, which is a percentage slightly higher than that noted in our study.\textsuperscript{16} In a similar retrospective Slovenian study of children under 2 years with bronchiolitis, RSV (57.5\%), HRV (25.6\%), and HBoV (18.4\%) were identified as the most common pathogenic viruses;\textsuperscript{17} their results were similar to ours in the case of RSV but higher in the case of HRV.

The current study indicated that a single viral infection (72.9\%) was most common type of infection in children under 2 years of age with bronchiolitis. We noted that RSV was the most common virus isolated, especially in infants under 6 months of age. We also noted that the percentage of RSV infection gradually decreased with age, which suggests that younger infants are more vulnerable to RSV disease; this is consistent with the findings of previous studies.\textsuperscript{18,19} A retrospective cohort study indicated that the reduced exposure of pregnant women to RSV epidemic contributed to more severe RSV-induced bronchiolitis in children under 6 months of age.\textsuperscript{20} Therefore, RSV-induced bronchiolitis is common in 6 months age; this may be associated with the circulation of antibodies that are not associated with RSV infection during pregnancy. Further investigations are necessary to identify specific susceptibility factors.

Further, in our study, MP was isolated in 10.8\% of our patients. A 2-year prospective study showed that 9.0\% of the bronchiolitis in infants had a positive pathogen of MP.\textsuperscript{21} Some researchers from Thailand have also reported the MP infected rate of 8.2.\textsuperscript{22} These are consistent with our study. We
also found bronchiolitis with single MP infection had a smaller chance of severe conditions like tachypnoea, dyspnea, need of oxygen and PICU admission. 1% of MP was detected in a multicenter study of children with severe bronchiolitis. A study also found that Children infected with CP and MP had less severe bronchiolitis than those infected with RSV. Therefore, we can speculate that MP infection has a lesser chance of causing severe bronchiolitis and explore this conjecture further.

One hundred and thirty seven (16.3%) of our patients had infection due to two pathogens. Interestingly, the distribution of multiple viral coinfection and viral-bacterial infection was similar. The probability of co-infection in children ≤ 6 months old was significantly lower than that in children aged between 6 months and ≤ 2 years of age. A prospective study from Turkey identified that the rate of coinfection among children with acute bronchiolitis was 34.2%, which is higher than the percentage observed in our study. Similarly, a study from Israel showed that the rate of co-infection in infants with acute bronchiolitis was nearly 40%, which is also higher than that in our study. A UK study reported an even higher percentage of 46%. The discrepancies in the rate of co-infection in bronchiolitis may be attributed to differences in the pathogen detection methods and the type of pathogens isolated.

The impact of co-infection on the severity of bronchiolitis still remains questionable. A comprehensive review in London revealed that multiple viral infection was associated with the admission of infants to the pediatric intensive care unit for the management of severe bronchiolitis. In contrast, some studies have shown that there is no correlation between the presence of multiple co-infections and severity of bronchiolitis. A Taiwanese study also reported that different viral pathogens do not give rise to different clinical characteristics among children with bronchiolitis. However, a Brazilian study revealed that both co-infection and RSV load influenced the prognosis of acute bronchiolitis in infants. Our findings in this study indicated that the duration of symptoms and duration of hospitalization in cases of single virus infection were significantly less than those observed in case of combined viral and MP infection. Further, single virus infection was least likely to induce fever. Thus,
we believe that co-infection can aggravate the disease. Further studies are necessary to confirm these associations.

Some of the most common reasons for admission due to bronchiolitis are hypoxia, requirement for supplemental oxygen, poor feeding, and respiratory disease.\textsuperscript{32-34} Small airway obstruction and the resultant mucus plugs and edema are believed to play a crucial role in the pathogenesis of bronchiolitis. In our study, the requirement of oxygen was most frequent among patients having co-infection with both viral pathogens and MP and least frequent among those with single MP infection. Studies have shown that the high incidence of hypoxia associated with RSV infection may be predictive of a poorer outcome,\textsuperscript{35} which is consistent with our findings.

Limitations
In our study, we included only patients with bronchiolitis who had undergone investigations for the detection of the pathogenic agents; among the patients, we did not enroll any patients with only bacterial infection. Immunofluorescence assays have variable and lower sensitivity (69.4\%) compared with PCR. Further investigations that cover a wider range of clinical presentations are warranted.

Conclusion
RSV was identified as the most common pathogen causing bronchiolitis in infants and young children, followed by MP and HRV. Co-infection with multiple pathogens leads to persistence of the disease for a longer period and increased severity of the symptoms. In particular, co-infection increases the risk of hypoxemia. Measures to increase awareness among healthcare personnel regarding the disease and its complications are necessary.

Abbreviations
PCR: Polymerase chain reaction; MP: Mycoplasma pneumoniae; RSV: Respiratory syncytial virus; CP: Chlamydophila pneumoniae; DFA: Direct immunofluorescence assay; IV: Influenza virus; PIV: Parainfluenza virus; ADV: Adenovirus; hMPV: Human metapneumovirus; HRV: Rhinoviruses; WBC: White blood cell; CRP: C-reactive protein; HBoV: Human bocavirus; SD: Standard deviation

Declarations
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**Author’s contributions**

JHT and JFW wrote the main manuscript text. WJJ and LH conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript. WJ and YDY carried out the initial analyses, reviewed and revised the manuscript, and approved the final manuscript. MJW and XJS did the microbiological detection. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The study was approved by the Medical Ethics Committee of Children’s Hospital of Soochow University. The parents of all study participants gave written informed consent before study enrollment.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Figures
Figure 1

Pathogen detection among children hospitalized due to bronchiolitis. RSV indicates respiratory syncytial virus, HRV indicates human rhinovirus, MP indicates M. pneumoniae, Boca indicates bocavirus, PIV indicates parainfluenza.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Availability of data and materials.xlsx