Abstract

Objectives: It is not clearly established if co-infections are more severe than single viral respiratory infections. The objective of this study was to study and to compare single infections and viral co-infections of respiratory syncytial virus (RSV) and Human Rhinovirus (HRV) in hospitalized children in Suzhou.

Patients and Methods: From December 2012 to December 2015, a prospective study was conducted for patients admitted with respiratory infection to the Pediatric Respiratory Department of the Children’s Hospital of Soochow University. Specimens of nasopharyngeal aspirate were taken for virological study by using polymerase chain reaction, and clinical data was recorded. Single RSV and HRV infections were selected and compared with mixed infections of RSV with HRV. Season of occurrence, gender, age distribution, duration of hospitalization, clinical signs; symptoms, diagnosis, blood culture, laboratory findings and other supplementary examinations were evaluated and compared among the three study groups.

Results: In this study 1177 cases were analyzed. Single infections (587 RSV and 560 HRV) were compared with 30 RSV-HRV mixed infections.

Conclusion: Co-infections between RSV-HRV are not very frequent. Overall viral co-infections do not present greater severity, but have mixed clinical features. No significant differences in the admission diagnosis, laboratory parameters, patient demographics and treatment measures between the two viral causes of respiratory illness were found. No correlation between viral load and disease severity was observed.

Keywords: Pneumonia, Co-infection, Children, Respiratory syncytial virus and Human rhinovirus.

INTRODUCTION

Acute respiratory tract infections (ARTIs) are common diseases among children and a major cause of hospitalization, mainly in infants. Viral pathogens play an important role in infants who present ARIs, respiratory syncytial virus (RSV) being the most important virus associated \(^{[1]}\). In addition, children with RSV infections are also exposed to a variety of other respiratory viruses with a similar seasonal pattern, mainly during winter months, such as influenza, human rhinovirus (HRV/ RV), human metapneumovirus (hMPV), and Human Boca virus (HBoV)\(^{[2-4]}\). Despite the fact that numerous studies have revealed that an important number of ARTI pediatric patients become simultaneously infected with multiple respiratory viruses, there are few studies focused on analyzing viral co-infections. This issue usually becomes a marginal part of the studies.

Over the past few years, several groups, including ours, have described various viral co-infection combinations compared with single ones, with different methodologies, and some of them observed worse prognosis with multiple viral infections, whereas others revealed a very similar prognosis for single virus infections.

Thus, there is a need of a carefully designed study to shed some light on this issue. We aimed to compare, in a prospective study, clinical characteristics and severity of
single versus viral co-infections, defined as simultaneous
detection of RSV with HRV, in a large cohort of
hospitalized children.

METHODS AND PATIENTS

Study site

The study was conducted at the Children’s Hospital of
Soochow University in Suzhou City, Jiangsu, China.
Suzhou, formerly Romanized as Soochow, is a major city
located in southeastern Jiangsu Province of East China,
about 100 km (62 mi) northwest of Shanghai. Its urban
population grew at an unprecedented rate of 6.5% between
2000 and 2014, which is the highest among cities with more
than 5 million people. Suzhou has a four-season, monsoon-
influenced humid subtropical climate with hot, humid
summers and cool, cloudy, damp winters with occasional
snowfall. Northwesterly winds blowing from Siberia during
winter can cause temperatures to fall below freezing at
night, while southerly or southwesterly winds during the
summer can push temperatures above 35 °C (95 °F). The
hottest temperature recorded since 1951 was at 41.0 °C
(106 °F) on 7 August 2013 and the lowest at −9.8 °C (14 °F)
on 16 January 1958. These different seasons affect the
activity of different bacterial and viral infections. The
Children’s hospital affiliated to Soochow University has
400 beds with an average yearly admission of about 10,000
children in the age group of 0-14 years. This hospital is also
a pediatric referral center for the Greater Suzhou Area and
the surrounding region. There are 5.26 million people in
Suzhou itself with a pediatric population (0-14 years) of 0.9
million[^5].

Study patients and case definitions

This is a prospective observational study. Ethics Committee
of Soochow University approved the study and the parents
of all children gave informed consent. The study population
comprised of all children less than 14 years of age with
ARTI admitted to the Children’s hospital of Soochow
University between December 2012 and December
2015. Patients assessed to have ARTIs requiring
hospitalization were recruited for participation in the study.
“Upper respiratory tract infection” (URTI) was diagnosed in
patients with rhinorrhea and/or cough and no signs of
wheezing, dyspnea, crackles, or bronchodilator use, with or
without fever. “Asthma” was diagnosed on the basis of the
National Asthma Education and Prevention Program
guidelines[^6]. All other episodes of acute expiratory
wheezing were considered to be “recurrent wheezing.”
Acute expiratory wheezing was considered to be
“bronchiolitis” when it occurred for the first time in children
aged less than 2 years. Cases with both focal infiltrates and
consolidation in chest radiographs were, in the absence of
wheezing, classified as “pneumonia.” However, if wheezing
were present, even though there was a radiographic
infiltrate, the patient was classified as having episodes of
wheezing.

Guidelines for diagnosis of severe pneumonia: Diagnosis of
severe pneumonia was made with reference to British
Thoracic Society Guidelines for the Management of
Community Acquired Pneumonia in Childhood[^7]. The
guidelines for infants included temperature >38.5°C, >70
breaths/min, moderate to the severe recession, nasal flaring,
cyanosis, intermittent apnea, grunting respiration, and not
feeding. For older children guidelines included temperature
>38.5°C, >50 breaths/min, severe difficulty in breathing,
nasal flaring, cyanosis, and grunting respiration. Indications
for admission to the ICU included the patient failing to
maintain a SaO2 of >92% in FiO2 of >0.6, the patient in
shock, rising respiratory and pulse rates with clinical
evidence of severe respiratory distress and exhaustion with
or without a raised arterial carbon dioxide tension (PaCO2),
and recurrent apnea or slow irregular breathing. The patients
with the following conditions should be excluded: preterm
children, those with heart diseases, chronic pulmonary
diseases, congenital airway malformations, known immune-
deficiencies and children with missing data were excluded
from the study. Asthma or recurrent wheezing was not
considered an underlying chronic disease

Clinical Assessment

Clinical characteristics of patients were analyzed. During the
hospital stay, and as part of the hospital’s medical record, a
physician filled out a study questionnaire with the following
variables: age, sex, date of admission, duration of stay, chief
complain; presence of cough, wheezing, nasal discharge, fever, duration of fever, peak temperature, clinical diagnosis, birth history, history of prematurity and underlying chronic diseases, need for oxygen therapy, evaluated via transcutaneous oxygen saturation, axillary temperature (≥38°C), presence of infiltrates and/or atelectasis in chest radiographs, administration of antibiotic therapy, length of hospital stay, total white blood cell (WBC) count, C-reactive protein (CRP) serum levels, and blood culture results (for those cases in which such tests had been performed).

Radiological assessment
Antero-posterior and lateral chest radiographs were taken upon admission. Interpretation was performed by a designated radiologist trained in the standard interpretation of chest radiographs for the diagnosis of childhood pneumonia.

Sample collection
Nasal aspirate samples were obtained from each patient within 24 hours of admission using a sterile plastic catheter that was introduced into the lower part of the pharynx via the nasal cavity. The samples were then divided into two aliquots for the detection of common viruses.

Detection of virus
Diagnosis of virus in the nasopharyngeal secretions of the virus common respiratory viruses detected by direct immunofluorescence, that is respiratory syncytial virus (respiratory syncytial virus, RSV), Adenovirus (adenovirus, ADV), Influenza virus A, B type (influenza virus type A and B, Inf-A and Inf-B), Parainfluenza virus and 1, 2, 3-type (Parainfluenza virus type 1~3). A sensitive quantitative PCR method targeted a 210-bp region from the conserved 5’ untranslated region (5’ UTR) was used to screen for HRV modified from a previous study. [8]

Laboratory tests
For each patient white blood cells (WBC) count was measured in an automated cell counter and the erythrocyte sedimentation rate (ESR) was measured in a period of one hour; C-reactive protein (CRP) was estimated by nephelometry (Image Beckmann). ESR <20 mm/hour and CRP < 0.5 mg/dl were considered at normal levels, while normal WBC range in the various age groups was considered to be in a range of 4-10 x10^{9}/L.

Statistical analysis
The continuous variables were compared using the Student t test if the data were abnormal in distribution. Three samples of continuous variables were compared using the ANOVA test. Categorical data were analyzed using the chi-squared (χ^2). Statistical analysis was performed using SPSS 17.0 statistical software. P < 0.05 was considered statistically significant

RESULTS
Season of occurrence
From the 587 patients with RSV infection; 325 cases, 55.3% were seen during winter; 88 cases, 14.99% were seen during spring; 16 cases, 2.72% were seen during summer and 158 cases, 26.91% were seen during autumn. While in the 560 patient with HRV infection 64 cases, 11.42% were seen in winter; 149 cases, 25.38% were seen in spring; 168 cases, 30.00% were seen in summer and 179 cases, 31.96% were seen in autumn. And in the 30 patients with co-infections of RSV + HRV 15 cases, 50.00% were seen in winter; 3 cases 10.00% were seen in spring; 1 case, 3.33% was seen in summer and 11 cases, 36.6% were seen in autumn. This shows that RSV is more predominant in winter and autumn where as HRV is more predominantly seen in autumn, spring and summer.
Baseline characteristics of the study population

A total of 1177 children with severe pneumonia were screened in this study. Three groups were created as 587 patients with RSV infection, 560 patients with HRV and 30 patients with viral co-infection of RSV+HRV. The proportion of male: female subjects with RSV infection are 382:205, with 382 male 65.07% and 205 female cases 34.9%. The proportion of male: female subjects with HRV infection are 346:214, with 346 male 61.78% and 214 female cases 38.2%. The proportion of male: female subjects with co-infection of RSV and HRV are 22:08, with 22 male 73.33% and 8 female cases 26.66%. The youngest was <1-month-old, while the oldest was 11.5-year-old. The patients were stratified in four age groups <1 year olds, 1-<3 years olds, 3-<5 years olds and ≥5-year-olds. The duration of hospitalization of patients with RSV, HRV and co-infections of RSV + HRV infections was from 3~30 days, 1~42 days and 4~20 days respectively and the average length of stay was 8.3±3.1 days, 7.9±3.3 days and 8.2±3.3 days respectively.

RSV infection was found to be significantly higher in patients in the age group < 1 year olds while HRV infection was found to be significantly higher in patients in the age groups older than 1 year old (P<0.05, respectively). There was no significant difference found in the duration of hospitalization between the three study groups. (P>0.05)
**Figure 2: Age distribution**

**Table 1: Duration of hospitalization, Gender and Age distribution**

<table>
<thead>
<tr>
<th>Variables</th>
<th>RSV* (n=587)</th>
<th>HRV* (n=560)</th>
<th>RSV+HRV (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/ Female, n</td>
<td>382/205</td>
<td>346/214</td>
<td>22/08</td>
<td>0.276</td>
</tr>
<tr>
<td>&lt;1 year old</td>
<td>447</td>
<td>277</td>
<td>21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1-&lt;3 years old</td>
<td>104</td>
<td>163</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3-&lt;5 years old</td>
<td>31</td>
<td>72</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥5-year-old</td>
<td>5</td>
<td>48</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of hospitalization, d, X ± SD**</td>
<td>8.3±3.1</td>
<td>7.9±3.3</td>
<td>8.2±3.3</td>
<td>0.067</td>
</tr>
</tbody>
</table>

*RSV denotes respiratory syncytial virus and HRV denotes human rhinovirus.

**SD denotes standard deviation.

**Clinical manifestations**

**Clinical symptom:**

From the 587 patients with RSV infection in children; 583 cases, 99.3% had cough; 387 cases, 65.90% had wheezing; 263 cases, 44.80% had nasal congestion; 201 cases, 34.2% had fever; 31 cases, 5.30% had dyspnea; 22 cases, 3.6% had feeding difficulties and 55 cases, 9.40% had gastrointestinal symptoms. While from the 560 patients with HRV in children 543 cases, 97.0% had cough; 258 cases, 46.10% had wheezing; 229 cases, 40.90% had nasal congestion; 242 cases, 43.20% had fever; 17 cases, 30.40% had dyspnea; 16 cases, 2.7% had feeding difficulties and 54 cases, 9.60% had gastrointestinal symptoms. In the 30 patients with coinfection of RSV + HRV 28 cases, 98.00% had cough; 30 cases, 100% had wheezing; 10 cases, 33.30% had nasal congestion; 10 cases, 33.30% had fever; 1 case, 3.30% had dyspnea; 1 case, 3.125% had feeding difficulties and 6 cases, 20.00% had gastrointestinal symptoms.
Clinical signs:
From the 587 patients with RSV infection in children 115 cases, 19.60% had tachypnea and 25 cases, 4.30% had cyanosis. In the 560 patients with HRV infection in children 70 cases, 12.50% had tachypnea and 13 cases, 2.3% had cyanosis. While in the 30 patients with co-infection of RSV+ HRV 4 cases, 13.30% had tachypnea and 0 cases, 0% had cyanosis.

Wheezing, nasal congestion and tachypnea were found to be significantly higher in patients with RSV infection as compared to those with HRV infection and co-infection of RSV+ HRV (P<0.05, respectively). While fever and dyspnea were found to be significantly higher in patients with HRV infection as compared to those with RSV infection and co-infections of RSV+HRV (P<0.05, respectively). Cough was found to show no significant difference between the three study groups (P>0.05).

Table 2: Clinical signs and symptoms

<table>
<thead>
<tr>
<th>Clinical symptoms (%)</th>
<th>RSV* (n=587)</th>
<th>HRV* (n=560)</th>
<th>RSV+HRV (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>583(99.3)</td>
<td>543(97.00)</td>
<td>28(98.00)</td>
<td>0.1</td>
</tr>
<tr>
<td>Wheezing</td>
<td>387(65.90)</td>
<td>258(46.10)</td>
<td>30(100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nasal Congestion/Runny Nose</td>
<td>263(44.80)</td>
<td>229(40.90)</td>
<td>10(33.30)</td>
<td>0.235</td>
</tr>
<tr>
<td>Presence of Fever</td>
<td>201(34.2)</td>
<td>242(43.20)</td>
<td>10(33.30)</td>
<td>0.006</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>31(5.30)</td>
<td>17(30.40)</td>
<td>1(3.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feeding Difficulties</td>
<td>22(3.6)</td>
<td>16(2.7)</td>
<td>1(3.125)</td>
<td>0.71</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>55(9.40)</td>
<td>54(9.60)</td>
<td>6(20.00)</td>
<td>0.159</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Signs, n (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tachypnea</td>
<td>115(19.60)</td>
<td>70(12.50)</td>
<td>4(13.30)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>25(4.30)</td>
<td>13(2.3)</td>
<td>0(0)</td>
<td>0.107</td>
</tr>
</tbody>
</table>

*RSV denotes respiratory syncytial virus and HRV denotes human rhinovirus.
Laboratory tests

In the 587 patients with RSV infection, the mean peripheral white blood cell count was $9.4 \pm 4.0 \times 10^9/L$, the mean percentage of granulocytes was $36.4 \pm 27.0\%$, lymphocytes was $55.4 \pm 16.6\%$, mean platelets level was $385.7 \pm 118.0\%$, eosinophils was $4.9 \pm 14.1 \times 10^9/L$ and Creatine Kinase Myocardial Band (CKMB) was $6.1 \pm 2.8 \text{ ng/mL}$. While in the 560 patients with HRV infection, the mean peripheral white blood cell count was $12.2 \pm 6.4 \times 10^9/L$, the mean percentage of granulocytes was $45.0 \pm 21.3\%$, lymphocytes was $46.4 \pm 20.0\%$, mean platelets level was $383.7 \pm 129.8\%$, eosinophils was $13.4 \pm 27.4 \times 10^9/L$ and CKMB was $16.7 \pm 15.3 \text{ ng/mL}$. Finally for the 30 patients with co-infections of RSV and HRV, the mean peripheral white blood cell count was $9.3 \pm 3.9 \times 10^9/L$, the mean percentage of granulocytes was $35.7 \pm 15.8\%$, lymphocytes was $55.7 \pm 16.2\%$, mean platelets level was $390.6 \pm 123.8\%$, eosinophils was $6.6 \pm 19.3 \times 10^9/L$ and CKMB was $11.9 \pm 12.6 \text{ ng/mL}$. No significant difference was found in CRP values because CRP is not so elevated in viral infections. In this study, the mean alanine aminotransferase (ALT) level of 587 patients with RSV infection was $32.19 \pm 29.6 \text{ U/L}$; the mean aspartate aminotransferase (AST) level was $51.2 \pm 28.3 \text{ U/L}$. Where as the mean ALT level of 560 patients with HRV was $27.8 \pm 53.0 \text{ U/L}$ and the mean AST level was $43.3 \pm 30.1 \text{ U/L}$. Finally the mean ALT level of 30 patients with RSV + HRV infection was $28.0 \pm 16.2 \text{ U/L}$ and the mean AST level was $43.9 \pm 13.3 \text{ U/L}$. Statistical differences in the levels of IgA, IgG and IgM were observed between the three groups.

In patients with HRV infection WBC, GRN% and eosinophils were significantly higher compared to those with RSV infection and co-infection of RSV and HRV (P<0.05, respectively). While in patients with RSV infection the percentage of lymphocytes, CKMBand AST levels were significantly higher compared to those with HRV infection and co-infection of RSV and HRV. (P<0.05, respectively).

Table 3: Laboratory tests- Blood analysis

<table>
<thead>
<tr>
<th>Laboratory tests (Blood Analysis, mean ±SD*)</th>
<th>RSV* (n=587)</th>
<th>HRV*(n=560)</th>
<th>RSV+HRV (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC*, x10^9/L</td>
<td>9.4±4.0</td>
<td>12.2±6.4</td>
<td>9.3±3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GRN*%</td>
<td>36.4±27.0</td>
<td>45.0±21.3</td>
<td>35.7±15.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L*, x10^9/L %</td>
<td>55.4±16.6</td>
<td>46.4±20.0</td>
<td>55.7±16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PLT*, x10^9/L</td>
<td>385.7±118.0</td>
<td>383.7±129.8</td>
<td>390.6±123.8</td>
<td>0.068</td>
</tr>
<tr>
<td>Eosinophils, x10^4/9/L</td>
<td>4.9±14.1</td>
<td>13.4±27.4</td>
<td>6.6±19.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST*, U/L</td>
<td>51.2±28.3</td>
<td>43.3±30.1</td>
<td>43.9±13.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT*, U/L</td>
<td>32.19±29.6</td>
<td>27.8±53.0</td>
<td>28.0±16.2</td>
<td>0.215</td>
</tr>
<tr>
<td>CKMB*, ng/mL</td>
<td>22.25±21.9</td>
<td>16.7±15.3</td>
<td>11.9±12.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG*, mg/dL</td>
<td>6.1±2.8</td>
<td>7.3±2.8</td>
<td>7.5±3.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4: Diagnosis.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>RSV* (n=587)</th>
<th>HRV*(n=560)</th>
<th>RSV+HRV (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial Pneumonia</td>
<td>477(81.30%)</td>
<td>431(77.00%)</td>
<td>25(83.30%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>11(1.90%)</td>
<td>5(0.90%)</td>
<td>0(0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>13(2.20%)</td>
<td>15(2.70%)</td>
<td>0(0%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Asthma</td>
<td>12(2.00%)</td>
<td>62(11.10%)</td>
<td>0(0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>13(2.1%)</td>
<td>379(67.70%)</td>
<td>5(16.70%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Days before admission, d, X ± SD</td>
<td>2.2±2.7</td>
<td>2.7±3.6</td>
<td>3.3±3.6</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*RSV denotes respiratory syncytial virus and HRV denotes human rhinovirus.
DISCUSSION

The United Nations Children’s Fund (UNICEF) and World Health Organization (WHO) have identified pneumonia as the major “forgotten killer of children”[9]. According to the WHO, about 1.9 million children worldwide die each year from respiratory tract infection and its complications [10]. Respiratory tract infection has been listed as the second leading cause of death in children under five [11]. It’s the leading cause of mortality among children less than five years of age, despite effective vaccines and nutritional and environmental interventions [12]. Viruses are key pathogens of respiratory infections [13,14]. Characterization of respiratory viruses and understanding its relationships with gender, age, and season will help to carry out the prevention and treatment of childhood respiratory tract infections and indirectly reduce the antibiotic abuse in clinical settings. Pneumonia is an illness, usually caused by bacterial, viral or more rarely fungal organisms. Common symptoms in children and infants include difficulty breathing, cough, and wheezing. Diagnosis involves confirmatory chest radiography and laboratory tests. Antibiotics are the preferred choice for treatment and management. Risks factors include low paternal education, low birth weight, and lack of breastfeeding. Key strategies for the prevention of childhood pneumonia are community –based case management, adequate nutrition and zinc intake [8].

We choose to study about RSV and HRV infections, as these are the most frequent viruses detected in pediatric outpatients and inpatients not just in China but also around the world as was found during a 12-year period research done in Turkey by Candan Çiçek [15]. These two viruses were found to be the two most predominant respiratory viral pathogens in our hospital. This result is consistent with the findings in most studies conducted in China such as in Kunming, Beijing and Chongqing [16] and also abroad [17-20] in United States [21], Brazil [22], and the United Kingdom [23]. Thus we know pneumonia is one of the leading causes of illness and death in children younger than 5 years of age worldwide, but there seems to be little information on the viral etiology of severe pneumonia in the developing countries, where the disease burden is particularly high. In this study we aimed at underscoring the importance of viral etiology in pediatric patients.

In Suzhou, one or more viruses could be identified in almost all the children suffering from pneumonia. One of the finding in this study was that RSV could be detected all year
round, peaked in November [Fig1]; this result was inline with the reports from Changsha, China [24]. The epidemiology of RSV and HRV varied by areas and seasons of the year[25]. One study showed no seasonality[26,27], whereas others found HRV peaking in spring and autumn[28, 29], which might have been, related to different rhinoviral species in different regions and seasons. On the other hand, this three-year study showed HRV peaking in autumn, spring and summer. In contrast, RSV presented with a seasonal pattern, predominantly in the winter months, which contributed to an increase in hospitalization during the winter. In our study we found only 30 cases positive for both RSV and HRV co-infection in a span of 3 years suggesting that these viruses are active during different seasons and hence it is an infrequent combination of pathogens causing severe pneumonia in children in Suzhou, China.

Our study has estimated the incidence of respiratory viruses among the hospitalized children with the diagnosis of ARTI that may be considered as viral etiology. It has also demonstrated the importance of RSV and HRV among ARTIs. In our study co-infection of RSV and HRV was the most significant viral infection associated with pediatric hospitalizations for Bronchial pneumonia (83.3%), followed by RSV single viral infection (81.3%) as compared to single HRV infection, especially in patients under one year of age, similar to findings in other studies [4, 30,31]. The possible reason is that younger children are more prone to be sick than older children due to their developing immune system and that parents are more likely to take their younger children to the doctor if they are sick. However HRV was detected in a larger proportion of hospitalized children older than one year of age [Fig 2]. From the above discussion we can say that in patients with bronchial pneumonia HRV is significantly less detected compared to RSV and co-infection of RSV and HRV (P<0.05). And in patients with asthma, and upper respiratory tract infection HRV is significantly more detected compared to RSV and co-infections of RSV and HRV (P<0.05,respectively)[Table 4]. No significant difference was found in the duration of hospitalization between the three study groups.

As expected, RSV was the main cause of wheezing; 387 of 587, 65.9% and also wheezing caused due to HRV was identified in a considerable number of patients; 258 of 560, 46.10% a finding comparable with the report of Miller[29]. Similar to other studies, RSV was associated with high severity of diseases. Cough, 99.3%; wheezing, 65.9%; Nasal congestion, 44.8%; fever, 34.2%; dyspnea, 5.3%; feeding difficulties, 3.6%; gastrointestinal symptoms, 9.4%; tachypnea, 19.5% and cyanosis, 4.3% were the most commonly encountered symptoms in hospitalized children with RSV [Table 2]. Wheezing, nasal congestion and tachypnea were found to be significantly higher in patients with RSV infection as compared to those with HRV infection and co-infection of RSV + HRV (P<0.05, respectively). While fever and dyspnea were found to be significantly higher in patients with HRV infection as compared to those with RSV infection and co-infections of RSV + HRV (P<0.05, respectively). Cough was found to show no significant difference between the three study groups (P>0.05), consistent with other studies. Leukocytosis may also occur in viral infections, as more than half of the RV-infected patients presented with leukocytosis, notably higher than that of RSV-infected patients [Table 3]. Previous studies had similar findings [2,4]. Whether these RV infections were accompanied by bacterial infection was uncertain. As suggested in other studies [2, 4, 34,35], in our study patients with HRV infection the GRN% and eosinophils counts were significantly higher compared to those with RSV infection and co-infection of RSV and HRV (P<0.05, respectively). While in patients with RSV infection the percentage of lymphocytes, CKMB and AST levels were significantly higher compared to those with HRV infection and co-infection of RSV and HRV (P<0.05, respectively).

LIMITATIONS
This study was not without shortcomings. It should be noted that a major limitation of this study is that RSV diagnosis was performed by antigen detection method while HRV diagnosis was by PCR, which might have underestimated
the prevalence of RSV. Second, the phylogenetic analysis of HRV was not performed, as the monthly distribution and clinical manifestations might differ among different HRV species. Thus, more work is essential for a better understanding of the seasonal epidemiology of different RV species. Only 30 patients with co-infection were studied, however, a larger study group is needed to learn more about virus co-infections.

CONCLUSION

Three important observations made in our study are: (1) no significant differences in clinical severity were observed between children with virus co-infection compared to those with single virus infection; (2) Single RSV infection was more predominate in children less than 1 year of age as compared to HRV which was more predominant in older children. (3) RSV presented with a seasonal pattern, peaking in the winter months in contrast to HRV infection, which was predominant in autumn, spring and summer.

In summary, our study suggests co-infections between RSV-HRV are not very frequent due to the difference in seasonal occurrence. Overall viral co-infections do not present greater severity, but have mixed clinical features. No significant differences in the admission diagnosis, laboratory parameters, patient demographics and treatment measures between the two viral causes of respiratory illness were found. No correlation between viral load and disease severity was observed.

ACKNOWLEDGMENTS

We thank all the physicians and nurses who helped enroll patients in the study and the staff that helped provide the necessary patient files from the medical archives of the Hospital. We also thank the staff members of the parasitology, physiology, and genetics laboratories for allowing us to use their facilities.

REFERENCES

5. Suzhou, From Wikipedia, the free encyclopedia