The Pathogens and Epidemiology of Community-Acquired Pneumonia among Children in Nanjing, China

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Abstract

Background

The roles of viral and atypical pathogens in respiratory tract infection were undervalued. The presented study explored the pathogens and epidemiology of community-acquired pneumonia (CAP) among children in Nanjing, China.

Methods

Serum samples of patients with CAP were tested with the Pneumoslide IgM kit from August 2011 to August 2013. The Pneumoslide IgM technology can simultaneously diagnose 9 main viral and atypical pathogens: *Legionella pneumophila* serogroup 1 (LP1), *Mycoplasma pneumoniae* (MP), *Coxiella burnetii* (COX), *Chlamydia pneumonia* (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV), Influenza A (INFA), Influenza B (INFB), Parainfluenza 1, 2 and 3 (PIVs). The data was analyzed by using Statistical Package for the Social Sciences for Windows (version 11.0, SPSS).

Results

Of a total of 1204 serum specimens tested 624 were positive. *M. pneumoniae* was the dominant pathogen, with INFB, PIVs, and RSV ranking second to fourth, respectively. The positive rates of MP, INFB, INFs and RSV were found to be associated with age, especially MP,
INFB and PIVs. The positive rates of MP, PIVs and RSV were also found to be associated with season. The positive rate of MP in autumn was the highest. The positive rates of LP1 in August and September, ADV in June and INFB in March were relatively higher than that in other months.

Conclusions

The results show there were 4 main viral and atypical pathogens causing CAP in our study. Some pathogens were found to be associated with age and season. *M. pneumoniae* was the most predominant pathogen among these 9 pathogens. It is necessary to take preventative measures in order to prevent the spread of these pathogens in susceptible age groups during peak season.

Key words: Community-Acquired Pneumonia; Pathogens; epidemiology; Pneumoslide IgM

Background

Community-acquired pneumonia (CAP) refers to pneumonia acquired outside of a health care facility. In the United States, CAP is the number-one cause of death from infection and the sixth leading cause of death overall. Each year, it is responsible for about 4.2 million outpatient visits and more than 60,000 deaths [1, 2]. It is important to understand the possible causes of CAP and which are most likely to occur so that
appropriate therapies can be selected. The primary pathogens responsible for CAP broadly include typical bacterial pathogens, atypical pathogens and viruses [3]. With the development of new laboratory testing technologies in recent years, it was found that the roles of viral and atypical pathogens in respiratory tract infection were undervalued. Research shows that about 33.3% of CAP cases are caused by viral and atypical pathogens [4]. Choi, et al. studied 198 patients with pneumonia and the determination was that 35% of the patients had a bacterial infection and 36% had viral infection [5]. In 2010, pneumonia was ranked in the United States as the sixth leading cause of death in children 1 to 4 years of age [6]. It is estimated that for every 1000 infants and children in North America and Europe, 35 to 40 will be affected by CAP [7]. Viral pathogens are the most common cause of CAP in children younger than 2 years of age, accounting for 80% of cases [8].

The prevalence of each pathogen varies from country to country and could be due to differences in seasons and geographic areas. Nanjing is the provincial capital of Jiangsu, and therefore the political economic and cultural center of the province. Nanjing is a metropolitan area with a population greater than 7 million people so to exploring the pathogens and epidemiology of pneumonia in Nanjing is significant for the health of children because of their weak immune defenses.
A rapid and standardized diagnostic method for the detection of pathogens in children with CAP is important. The currently validated methods to define the etiology of infection are serology, cell culture and PCR. Viral culture can also be employed for most of the respiratory viruses, but the need for specific culture medium and the lengthy diagnosis times are substantial disadvantages [4]. Recently, PCR has been reported as a rapid method with high sensitivity that may exceed that of culture, but PCR assays need specialized equipment and the reagents are expensive [9]. Much research shows that the Pneumoslide IgM test is a reasonably sensitive, highly specific, easy, rapid and cost-effective technique for detection of viral or atypical pathogens [10]. As an indirect immunofluorescence technique for IgM detection, Pneumoslide IgM can simultaneously diagnose 9 main pathogens of infectious disease of the respiratory tract, including the *Legionella pneumophila* serogroup 1 (LP1), *Mycoplasma pneumoniae* (MP), *Coxiella burnetii* (COX), *Chlamydophila pneumoniae* (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV), Influenza A (INFA), Influenza B (INFB), Parainfluenza 1, 2 and 3 (PIVs). Pneumoslide IgM could detect virus in 25% of patients, whereas viral culture detected in them 16.7%. Sally compared the technique with PCR, and reported the sensitivity and specificity of Pneumoslide IgM for RSV was 75 and 98.1%, respectively, whereas they were 78 and 95% for M. pneumoniae, respectively [10]. So
Pneumoslide IgM had reasonable sensitivity and specificity for detection pathogens causing CAP.

**Material and methods**

**Subject information**

The study was conducted on 1204 children patients suffering from CAP recruited from Zhongda Hospital, Southeast University, Nanjing, China. Of the patients, 715 children were male and 489 were female. Samples were collected from August 2011 to August 2013. Diagnosis of pneumonia followed World Health Organization Criteria (1994). From 4h after birth to 14 years old, children were divided into four groups: infants group (newborn ~1 year old), 184 cases; toddlers group (>1 ~3 years old), 477 cases; preschool group (>3 ~ 6 years old), 403 cases; school children group (7 ~ 14 years old), 140 cases. According to the seasons when the Pneumoslide IgM tests were performed, all the subjects were divided into four group: spring group (March, April and May), 316 cases; summer group (June, July and August), 295 cases; autumn group (September, October and November), 227 cases; winter group (January, February and December), 366 cases. The data was analyzed by using Statistical Package for the Social Sciences for Windows (version 11.0, SPSS).

**Blood sampling**
Four milliliters of whole venous blood was withdrawn from each child. The samples were centrifuged at 2000g for 10 min at 4°C. Serum was separated and stored at -20°C until assayed with the Pneumoslide IgM test.

**Pneumoslide IgM test** (Vircell-slide, Granada, Spain)

Each slide has 10 wells with each well containing one of the following antigens: *Legionella pneumophila* serogroup 1 (LP1), *Mycoplasma pneumoniae* (MP), *Coxiella burnetii* (COX), *Chlamydophila pneumoniae* (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV), Influenza A (INFA), Influenza B (INFB), Parainfluenza 1, 2 and 3 (PIVs) and a cell control. Serum samples were diluted 1:1 with phosphate buffered saline (PBS), and then treated with anti-human IgG sorbent. The sorbent-treated diluted serum was incubated 90 min at 37°C with the 10 slide wells. The slide was washed twice with PBS before the fluorescent secondary IgM antibody was added to the wells and incubated at 37°C for 30 minutes. The slide was then washed twice with PBS and the greenish-yellow fluorescent signal was detected with a fluorescence microscope (Zeiss, Oberkochen, Germany)

**Results**

**The positive rates of 9 pathogens**
Of a total of 1204 samples tested, 624 were positive for a positive rate of 51.83%. The most predominant pathogen was *M. pneumoniae*, with a positive rate of 40.78%. The pathogens ranking second to fourth place were INFA, PIVs, and RSV, with positive rates of 7.06%, 4.82%, and 3.32%, respectively. Among all of the samples, only 1 COX and 1 INFA infection were detected (Table 1).

Table 1. The positive rates of 9 pathogens

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Number</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma pneumonia</em> (MP)</td>
<td>491</td>
<td>40.78</td>
</tr>
<tr>
<td>Influenza B (INFB)</td>
<td>85</td>
<td>7.06</td>
</tr>
<tr>
<td>Parainfluenza 1, 2 and 3 (PIVs)</td>
<td>58</td>
<td>4.82</td>
</tr>
<tr>
<td><em>Respiratory syncytial virus</em> (RSV)</td>
<td>40</td>
<td>3.32</td>
</tr>
<tr>
<td>Adenovirus (ADV)</td>
<td>13</td>
<td>1.08</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em> serogroup 1 (LP1)</td>
<td>11</td>
<td>0.91</td>
</tr>
<tr>
<td><em>Chamydophila pneumonia</em> (CP)</td>
<td>4</td>
<td>0.33</td>
</tr>
<tr>
<td><em>Coxiella burnetii</em> (COX)</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>Influenza A (INFA)</td>
<td>1</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**The positive rates of pathogens isolated from different age groups**

For the 4 major pathogens, the positive rates of MP, INFB, PIVs and RSV were found to be associated with age, with p values of 0.000, 0.000, 0.000, and 0.038, respectively. *M. pneumoniae* was the most predominant pathogen in all age groups, compared to other pathogens. The positive rates of MP and INFB in preschooler group were 55.33% and 12.41%, which were the highest, compared to other age groups, but the positive rates of these two pathogens were just 13.04% and 2.17% in
infants group. The positive rate of PIVs in school children group was higher than that in other age groups and it was seen that the positive rates increased with the age of patients (0.54% in infants group; 1.68% in toddlers group; 8.19% in preschool group; 11.43% in school group) (Table 2).

Table 2. The positive rates of pathogens isolated from different age groups with Pneumoslide IgM test

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Infants (%)</th>
<th>Toddlers (%)</th>
<th>Preschooler (%)</th>
<th>School children (%)</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=184</td>
<td>N=477</td>
<td>N=403</td>
<td>N=140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MP</td>
<td>24 (13.04)</td>
<td>169 (35.43)</td>
<td>223 (55.33)</td>
<td>75 (53.57)</td>
<td>109.105</td>
<td>0.000</td>
</tr>
<tr>
<td>INFB</td>
<td>4 (2.17)</td>
<td>19 (3.98)</td>
<td>50 (12.41)</td>
<td>12 (8.57)</td>
<td>31.624</td>
<td>0.000</td>
</tr>
<tr>
<td>PIVs</td>
<td>1 (0.54)</td>
<td>8 (1.68)</td>
<td>33 (8.19)</td>
<td>16 (11.43)</td>
<td>40.923</td>
<td>0.000</td>
</tr>
<tr>
<td>RSV</td>
<td>11 (5.98)</td>
<td>19 (3.98)</td>
<td>7 (1.74)</td>
<td>3 (2.14)</td>
<td>8.450</td>
<td>0.038</td>
</tr>
<tr>
<td>ADV</td>
<td>4 (2.17)</td>
<td>5 (1.05)</td>
<td>3 (0.74)</td>
<td>1 (0.71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP1</td>
<td>0 (0.00)</td>
<td>6 (1.26)</td>
<td>4 (0.99)</td>
<td>1 (0.71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>4 (2.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX</td>
<td>1 (0.54)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INFA</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (0.71)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MP: Mycoplasma pneumonia; INFB: Influenza B; PIVs: Parainfluenza 1, 2 and 3; RSV: Respiratory syncytial virus; ADV: Adenovirus; LP1: Legionella pneumophila serogroup 1; CP: Chamydophila pneumonia; COX: Coxiella burnetii; INFA: Influenza A.

The positive rates of pathogens isolated from different seasons

Among the 4 major respiratory tract pathogens, the positive rates of MP, PIVs and RSV were found to be associated with seasons (MP, p=0.011; PIVs, p=0.009; RSV, p=0.038) but the positive rates of INFB were not found to be associated with season (p=0.063) (Table 3). The positive rates of MP were always high through the seasons (>29.63%) (Figure 1),
compared to the other 8 pathogens. The positive rates of MP were relatively high in summer and autumn (45.08% and 47.14% respectively) and relatively low in spring and winter (38.29% and 35.52% respectively) (Table 3). The positive rates of LP1 in August and September and of ADV in June were higher than in other months (Figure 2, 3).

Table 3 The positive rates of pathogens isolated from different seasons with Pneumoslide IgM test

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Spring (%)</th>
<th>Summer (%)</th>
<th>Autumn (%)</th>
<th>Winter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=316</td>
<td>N=295</td>
<td>N=227</td>
<td>N=366</td>
</tr>
<tr>
<td>MP</td>
<td>121 (38.29)</td>
<td>133 (45.08)</td>
<td>107 (47.14)</td>
<td>130 (35.52)</td>
</tr>
<tr>
<td>INFB</td>
<td>30 (9.49)</td>
<td>14 (4.75)</td>
<td>20 (8.81)</td>
<td>21 (5.74)</td>
</tr>
<tr>
<td>PIVs</td>
<td>23 (7.28)</td>
<td>12 (4.07)</td>
<td>15 (6.61)</td>
<td>8 (2.19)</td>
</tr>
<tr>
<td>RSV</td>
<td>14 (4.43)</td>
<td>3 (1.02)</td>
<td>6 (2.64)</td>
<td>17 (4.64)</td>
</tr>
<tr>
<td>ADV</td>
<td>4 (1.27)</td>
<td>6 (2.03)</td>
<td>1 (0.44)</td>
<td>2 (0.55)</td>
</tr>
<tr>
<td>LP1</td>
<td>2 (0.63)</td>
<td>4 (1.36)</td>
<td>3 (1.32)</td>
<td>2 (0.55)</td>
</tr>
<tr>
<td>CP</td>
<td>0 (0.00)</td>
<td>1 (0.34)</td>
<td>3 (1.32)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>COX</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (0.44)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>INFA</td>
<td>1 (0.32)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

MP: Mycoplasma pneumonia; INFB: Influenza B; PIVs: Parainfluenza 1, 2 and 3; RSV: Respiratory syncytial virus; ADV: Adenovirus; LP1: Legionella pneumophila serogroup 1; CP: Chamydophila pneumonia; COX: Coxiella burnetii; INFA: Influenza A.

The mixed infection among these respiratory tract pathogens

Of the 1204 samples tested, 79 were confirmed as mixed infection, for a 6.56% positive rate. Generally, there were four major mixed infection types. There were 35 samples infected with MP and INFB (44.30% positive rate) and 36.71% of these samples infected with MP and PIVs. Other mixed infection types were MP with RSV and INFB with PIVs.
The results show that patients infected with MP were susceptible to other infectious pathogens, resulting in mixed infections.

**Discussion**

Some pathogens may have a higher seasonal incidence than others in certain geographic areas and, therefore, the diagnosis of those most prevalent diseases would be of value. It was therefore important to know the epidemiology of pneumonia in Nanjing and it was important to know how the prevalence of the CAP-causing microorganisms is related to seasonal changes. Among children in Nanjing, there were 4 major viral and atypical pathogens causing CAP that were detected with the Pneumoslide IgM test. *M. pneumoniae* was the most predominant pathogen in different age and seasonal groups, which did not agree with previous results. Ren, *et al.* found that INF was the most predominant pathogen among the Beijing population [11], although this difference may result from the different geographic area and different population. MP is highly contagious and can spread between people through bodily fluids and airborne droplets from sneezing and coughing. It was most easily spread among people who were in close contact with one another [12]. In China, children go to daycare when they were about 3 years old and go to primary school when they are about 7 years old. Because the preschool and school children lived or studied in densely populated
(crowded) area, MP was most easily spread among children. Our results show a significant association among age group (p<0.01) (Table.2). The positive rates of MP in the preschool and school groups were higher than the infants or toddlers group. Although MP infections can occur at any time of year, they were most common in the late summer and autumn.

Our results confirmed the epidemiological characteristics of MP; the positive rates of MP in summer and autumn were 45.08% and 47.14%, respectively and MP was significantly associated with seasons (p<0.01) (Table.3). Patients with MP infection were susceptible to infection with other pathogens, resulting in mixed infections, such as MP with INFB or MP with PIVs. When treating a mixed infection, particular attention must be paid to the characteristics of the infection and the responsible pathogens.

Influenza is an RNA virus of the *orthomyxoviridae* family and has three serotypes (A, B, and C) which have been described [13, 14]. In the samples tested, only 1 INFA positive was detected. However 85 samples infected with INFB were detected in these same subjects (Table.1). INFB usually causes disease in populations confined to closed spaces, such as daycare centers and boarding schools [15]. Our results show the positive rates of INFB in the preschool and school children groups were higher than that in the infants and toddlers groups (p<0.01) (Table.2).
PIVs can spread from person to person through close contact [16, 17]. Like MP and INFB, children who lived in crowded area (such as daycare and boarding school conditions) were susceptible to infection with PIVs (p<0.01) (Table.2). People usually get infected with PIVs in the spring, summer, and fall. Our results were concordant with the characteristics of PIVs (p<0.01) (Table.3).

In the United States, 60% of infants are infected during their first RSV season, and nearly all children will have been infected with the virus by 2–3 years of age [18]. Our results show a higher association for RSV infection among the infants and toddlers groups (p<0.05), although the positive rates of RSV in the infants and toddlers groups were only 5.98% and 3.98, respectively (Table.2). RSV was found to be associated with season (p<0.05) and the positive rates of RSV were higher in the spring and winter seasons (Table.3).

**Conclusions**

As observed in our results, there were 4 major viral and atypical pathogens causing CAP, and some pathogens were found to be associated with age and season. *M. pneumoniae* was the dominant pathogen and easily infects patients suffering from other pathogens, resulting in mixed infection. Crowded spaces (daycare, boarding school, etc.) may be the major reason for the spread of MP, INFB, and INFs among children in
Nanjing, China. The study accumulated valuable data about the pathogens and epidemiology of CAP among children in Nanjing, not only to increase the knowledge of the epidemiological profiles of pneumonia pathogens, but also to plan better therapeutic and prevention strategies to prevent the spread of the pathogens in susceptible age groups during peak season.

Limitation

Pneumoslide IgM test has reasonable sensitivity and specificity for detection of pathogens; however the test could not detect bacterial pathogens. So the epidemiology profiles presented by the study were just for viral and atypical pathogens causing community-acquired pneumonia.

Competing interests

The authors declare that they have no competing Interests.

Authors’ contributions

KC and RJ conceived and designed the experiments. KC and YS prepared the serum and performed Pneumoslide IgM test. CY and LL analyzed the data. KC wrote the paper.

Acknowledgments

We are deeply appreciative of the study participants. The work was partly supported by the national natural science foundation of China (No.
References


Figure 1. The positive rates of MP from January to December. The positive rates of MP were always high through the seasons (>29.63%).

Figure 2. The positive rates of LP1 from January to December. The positive rates of LP1 in August and September were higher than in other months.

Figure 3. The positive rates of ADV from January to December. The positive rates of ADV in June were higher than in other months.
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