Original Article

Analysis of pathogenic and clinical characteristics of children with pertussis-like syndrome

Xiaojuan Lin1, Lili Zhong1, Yujiao Luo1, Zhongping Deng2, Liangyi Xie1, Fang Peng1, Han Huang1, Liping Liu1, Min Chen1, Danxia Peng1, Xiaofang Ding1, Manzhi Wang3, Haiying Han4, Ge Zhou5, Yuhui Huang6, Ping Jiang7

1Children’s Medical Center, Hunan Provincial People’s Hospital, Changsha 410005, Hunan Province, P. R. China; 2Sansure Biotech INC, Changsha 410205, Hunan Province, P. R. China; 3Changsha Central Hospital, Changsha, Hunan Province, P. R. China; 4Shaoyang Central Hospital, Shaoyang 422000, Hunan Province, P. R. China; 5The First People’s Hospital of Yueyang, Yueyang 414000, Hunan Province, P. R. China; 6Pediatrics of Maternal and Child Health Care Hospital of Ziyang District, Yiyang 413000, Hunan Province, P. R. China; 7The First Hospital of Hunan University of Chinese Medicine, Changsha 410005, Hunan Province, P. R. China

Received January 14, 2019; Accepted June 5, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: Objective: The aim of the current study was to investigate the bacterial spectrum and clinical features of children with pertussis-like syndrome. Methods: Sputum and nasopharyngeal swabs from children with pertussis-like syndrome in Hunan Province were collected from January 2015 to January 2018. Bacterial culturing and fluorescence quantitative polymerase chain reaction (qRT-PCR) analyses were conducted to detect Bordetella pertussis (BP), Mycoplasma pneumoniae (MP), Chlamydia pneumoniae (CP), Adenovirus (ADV), Respiratory syncytial virus (RSV), Human coronavirus (HCOV-229E, HCOV-OC43), Human bocavirus (HBoV), Human rhinovirus (HRV), Influenza virus A/B (Flu A/B), and Human parainfluenza virus 1-3 (HPIV 1-3). Retrospective analysis of clinical features and outcomes in children with positive pathogenic bacteria was performed. Results: A total of 280 cases were enrolled, with pathogens detected in 221 cases. The positive detection rate of BP was the highest (135/280, 48.2%). Of the positive patients, 143 cases were infected by a single pathogen, while 78 cases were found to have mixed infections. Cases aged 2-4 months old showed the highest frequency in both groups. No statistical differences were identified regarding sex, season, and vaccine un-inoculation rates between the two groups. Patients in the BP group were more likely to age less than 12 months. Cases in BP groups had facial flushing when coughing. They often coughed at night and had cock-like roars after coughing. Incidence rates of fever and lung wet rales of BP groups were significantly lower than those of non-BP groups. Conclusion: BP plays a vital role in pertussis-like syndrome in Hunan Province. Therefore, early diagnosis of BP is of great significance for clinical treatment.

Keywords: pertussis-like syndrome, pathogen, bordetella pertussis

Introduction

Pertussis, a respiratory infectious disease, seriously affects the growth and quality of life of children. Pertussis is caused by Bordetella pertussis (BP) infections. It is a major disease causing infant deaths [1, 2]. The main symptoms of pertussis include paroxysmal coughing and cock-like roars, with or without facial flushing, vomiting, cyanosis, and apnea after coughing. Poor treatment results and prolonged courses are the main traits of pertussis. Since 1940, pertussis has become a vaccine-preventable disease. Vaccination rates have increased with time. Incidence rates of pertussis have also declined [3-5]. However, incidence of pertussis has now increased again. There has been an outbreak of pertussis in local areas in recent years [6-9]. Pertussis-like syndrome refers to patients with clinical symptoms in accord with pertussis, but with a lack of laboratory detection evidence or with definite history of exposure to pertussis. Studies have shown that a variety of pathogens, such as Haemophilus influenza (HI), Streptococcus pneumoniae, Respiratory syncytial virus (RSV), Adenovirus (ADV), parainfluenza virus (HPIV), mycoplasma, and chlamydia, can cause pertussis-like syndrome, in addition to BP [10, 11]. Therefore, it is particularly important to identify pathogens of pertussis-like syndrome.

At present, many researchers use fluorescent real-time polymerase chain reaction (RT-PCR)
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analysis to detect various respiratory pathogens, including BP [12, 13]. This method is simple and easy to operate, with high clinical utility. However, this method has not been widely carried out in clinical work due to strict requirements for equipment and operators, as well as high costs.

In the current study, providing evidence for the prevention and treatment of children with pertussis-like syndrome, sputum culture and qRT-PCR methods were used to detect pathogens in sputum and nasopharyngeal aspirates (NPA) of children with pertussis-like syndrome, between January 2015 to January 2018 in Hunan, China. Retrospective analysis of clinical data was also conducted.

Methods

Patients

From January 2015 to January 2018, this study included children visiting the hospital, aged 14 or less than 14 years, with pertussis-like syndrome in the Hunan region of China (Hunan Provincial People’s Hospital, Changsha Central Hospital, Xiangya Hospital, Loudi Central Hospital, The First Affiliated Hospital of University of South China, First people’s Hospital of Shaoyang, Pediatrics of Maternal and Child Health Care Hospital of Ziyang District, Yiyang). Inclusion criteria: Paroxysmal coughing and cock-like roars after coughing, with or without facial flushing, vomiting, cyanosis, and apnea. Exclusion criteria: Patients older than 14 years; Patients with airway structural deformities; Patients with external pressure factors around the airway. Written informed consent was obtained from all parents.

Sample and medical records collection

Sputum and NPA of patients were collected within 48 hours after admission. Collected sputum samples were immediately sent to the Bacteriology Room. Collected NPA samples were stored in aseptic tubes containing 2 mL of physiological saline. They were then they were sealed and stored in the 40°C refrigerator. Every two weeks, the sterile tubes were sent to Hunan Sheng Xiang Biotechnology Co. Ltd. for detection.

Preparation of standard samples and primer probes

Standard samples of 14 kinds of pathogens were provided by Hunan Sheng Xiang Biotechnology Co. Ltd. Primers and probes were obtained from related references [14-23]. The final concentrations of primers and probes were 20 μM and 10 μM, respectively.

Sample processing and total nucleic acid extraction

The specimens were thawed at 37°C. They underwent concussion to obtain the liquid sample. Nucleic acid of the pathogens was extracted using the Magnetic Beads Nucleic Acid Extraction Kit (Hunan Sheng Xiang Biotechnology Co. Ltd), according to manufacturer instructions.

Detection of nucleic acid of the pathogens

The One-step RT-PCR Kit was used to detect 9 kinds of RNA viruses, including RSV, rhinovirus (RHV), IFVA-B, PIV1-3, human coronavirus 229E (HCoV-229E), and human coronavirus OC43 (HGoV-OC43). Taqman Universal Mater Mix II with UNG Reagent was used to detect 5 kinds of DNA pathogens, including ADV, MP, CP, HBoV, and BP. The correlation coefficient of the standard curve was $R^2 > 0.99$. The amplification efficiency (Eff %) was between 90% and 100% for experimental results. Fluorescence was detected as the last step of each cycle. Ct values were calculated using the instrument’s own software, with quantitative results recorded. Specimens with a pathogen load of ≥200 copies/μl were considered positive.

Statistical analysis

Experimental results were analyzed using SPSS18.0 (SPSS, Chicago, IL, USA). Moreover, $\chi^2$ tests, continuous correction $\chi^2$ tests, and t-tests were used to compare differences between the two groups. $P < 0.05$ indicates statistical significance.

Results

Distribution characteristics of detected pathogens

Of the 280 cases, pathogens were detected in 221 cases. The total detection rate of patho-
pertussis-like syndrome in children.  

The detection rate of the positive for pathogens was 78.9%. The BP detection rate (135/280, 48.2%) was the highest, followed by RSV (29/280, 10.4%), MP (24/280, 8.6%), HPIV3 (23/280, 8.2%), and ADV (20/280, 7.1%). Klebsiella pneumoniae and other pathogens were also detected. Results of pathogen detection are shown in Table 1.

Distribution characteristics of single or multiple pathogen detection

Of the 221 cases positive for pathogens, 143 cases (64.7%) were infected by a single pathogen. The top five single pathogens detected were BP (76 cases), RSV (23 cases), MP (20 cases), HPIV16 (16 cases), and ADV (9 cases). Of the 221 cases positive for pathogens, 78 cases (35.3%) showed mixed infections. Of the mixed infections, the number of BP mixed infections was the highest (59 cases, 75.6%), while non-BP mixed infections accounted for 19 cases (24.4%). BP + ADV (11 cases) was the most among mixed infections, followed by BP + HPIV3 (7 cases), BP + RSV (6 cases), BP + FluA (5 cases), BP + MP (4 cases), BP + Hemophilus influenzae (4 cases), and BP + RHV (4 cases).

Comparison of general material between the BP group and non-BP group

Cases infected by BP in the 143 cases of single pathogen infections were enrolled in the BP group (76 cases). The others were divided into the non-BP group (67 cases). Differences in clinical features between the BP group and non-BP group were compared.

There were no significant differences in the positive detection rate for males or females between the BP group and non-BP group ($\chi^2=1.410$, $P=0.235$) (Table 2). In the BP and non-BP group, most cases were aged 2-4 months. In the BP group, 71 cases were aged no more than 12 months, while 5 cases were aged more than 12 months. In the non-BP group, 53 cases were aged no more than 12 months, while 14 cases were aged more than 12 months. Compared with the non-BP group, the proportion of cases aged no more than 12 months was significantly higher in the BP group ($\chi^2=6.335$, $P=0.012$) (Figure 1). Vaccination rates of cases aged 3-12 months in the BP group and non-BP group were 52.6% and 49.3%, respectively. Un-vaccination rates of the two groups were high, but there were no significant differences ($\chi^2=1.032$, $P=0.310$) (Table 2). No significant differences were found in the exposure history of family members concerning respiratory tract infections between the two groups within 21 days (Table 2). Cases in BP and non-BP groups had onset in all four seasons. Higher incidence was found in summer and autumn seasons. There were no significant differences in the distribution of seasons, spring, summer, autumn, and winter, between the two groups. Results are shown in Table 3.

Comparison of clinical features of cases in the BP group and non-BP group

Results of comparisons of cough characteristics, cough patterns, and accompanying symp-
Pertussis-like syndrome in children

Table 2. Comparison of gender, age, vaccination rates, and exposure history of family members to respiratory tract infections within 21 days

<table>
<thead>
<tr>
<th></th>
<th>BP group (n=76)</th>
<th>Non-BP group (n=67)</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>39 (51.3%)</td>
<td>41 (61.2%)</td>
<td>1.41</td>
<td>0.235</td>
</tr>
<tr>
<td>Age (≤12 months)</td>
<td>71 (93.4%)</td>
<td>53 (79.1%)</td>
<td>6.335</td>
<td>0.012*</td>
</tr>
<tr>
<td>Vaccination rate</td>
<td>40 (52.6%)</td>
<td>33 (49.3%)</td>
<td>1.032</td>
<td>0.310</td>
</tr>
</tbody>
</table>

**Exposure history of family members of respiratory tract infections within 21 days**

<table>
<thead>
<tr>
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<td>0.310</td>
</tr>
</tbody>
</table>

BP: Bordetella pertussis. *: P < 0.05.

Table 3. Comparison of different season distributions of BP and non-BP groups

<table>
<thead>
<tr>
<th></th>
<th>BP group (n=76)</th>
<th>Non-BP group (n=67)</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>17 (22.4%)</td>
<td>10 (14.9%)</td>
<td>1.288</td>
<td>0.256</td>
</tr>
<tr>
<td>Summer</td>
<td>20 (26.3%)</td>
<td>23 (34.3%)</td>
<td>1.087</td>
<td>0.297</td>
</tr>
<tr>
<td>Autumn</td>
<td>31 (40.8%)</td>
<td>23 (34.3%)</td>
<td>0.632</td>
<td>0.426</td>
</tr>
<tr>
<td>Winter</td>
<td>8 (10.5%)</td>
<td>11 (16.5%)</td>
<td>1.073</td>
<td>0.300</td>
</tr>
</tbody>
</table>

BP: Bordetella pertussis.

Detection results for BP and non-BP groups suggest that increases in leukocytosis were more pronounced in the BP group, mainly lymphocytosis (P < 0.05). However, differences in imaging changes between the two groups were not significant (P > 0.05). Results are shown in Table 5.

Discussion

Although pertussis has become a preventable disease with the use of vaccines, incidence rates have increased, worldwide, in recent years [24-26]. Therefore, it is particularly important to improve detection methods of pathogenic bacteria, including BP, as soon as possible for children with pertussis-like syndrome. Symptoms include paroxysmal coughing and cock-like roaring, with or without facial flushing, vomiting, cyanosis, and apnea. Pathogen isolation has been considered the gold standard method for detection of BP. However, this method has high requirements for specimen collection, transportation, culture conditions, disease duration, and antibiotic use. These factors have led to low positive rates of clinical culturing, longer culture times, and unfavorable guidance to clinical treatment. Therefore, the current study used qRT-PCR to detect BP.

In this study, the total detection rate of pathogens of the 280 children with pertussis-like syndrome was 78.9%. Detected pathogens included bacteria, virus, mycoplasma, and chlamydia, indicating that infections with many pathogens can cause pertussis-like syndrome. Present results are consistent with previous studies [5, 10, 27, 28].
Concerning detection results, BP had the highest detection rate. BP was followed by RSV, MP, HPIV3, and ADV. Results suggest that these pathogens are the main pathogens causing pertussis-like syndrome in Hunan Province. Compared with different regions of China in the same period [29], the detection rate of BP was comparable. However, compared with a study performed by Ferrer et al. in 2000 [30], current data suggests that the prevalence of BP was significantly increased. Some viruses, such as RSV, HPIV3, and MP, accounted for a considerable proportion. The prevalence of pertussis-like syndrome caused by bacterial infections has decreased. However, the RT-PCR method may increase the detection rate of some pathogens. Of the 221 cases positive for pathogens, the detection rate of cases infected by a single pathogen was 64.7%. This indicates that pertussis-like syndrome caused by a single pathogen infection was common in clinic. Concerning mixed infections, BP mixed infections accounted for 75.6%. This suggests that mixed infections of pertussis-like syndrome are mainly caused by BP. The most common pathogen of BP mixed infections was ADV, followed by RSV, fluA, MP, Haemophilus influenza, and RHV. In a study performed by A. Frassanito et al. [30], they analyzed 53 infants admitted to the hospital for BP, including 9 infants infected with hRV, 3 infants with hCoV, 2 infants with RSV, and 2 infants with influenza. Because the number of cases of co-infections was not large, it was speculated that BP may be more likely to be associated with viral infections.

Comparisons of clinical features between the BP group and non-BP group were conducted. Results showed that cases in the BP group tended to be younger (≤12 months). Patients in the BP group had facial flushing when coughing. They often coughed at night, with a cock-like roar after coughing. However, incidence rates of fevers and lung wet rales of the BP group were significantly lower than those of the non-BP group. White cells and percentages of lymphocytes were higher, compared to the non-BP group. Conclusions drawn by some foreign studies have been controversial. A study performed by Jõgi P et al. [31] suggested that patients with positive BP had a higher percentage of inhaled roaring and vomiting after coughing. However, there were no statistical differences, compared with the negative group. Studies conducted by Gökçe et al. [32] and Al Maani A et al. [33] also suggested that there was no statistical differences in clinical symptoms between pertussis and non-pertussis groups. However, the increased percentage of

Table 4. Comparison of clinical features of cases in the BP group and non-BP group

<table>
<thead>
<tr>
<th>Feature</th>
<th>BP group (n=76)</th>
<th>Non-BP group (n=67)</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red face at cough</td>
<td>58 (76.3%)</td>
<td>40 (59.7%)</td>
<td>4.558</td>
<td>0.033*</td>
</tr>
<tr>
<td>Vomiting after cough</td>
<td>25 (32.9%)</td>
<td>22 (32.8%)</td>
<td>0.000</td>
<td>0.994</td>
</tr>
<tr>
<td>Cock-like roar</td>
<td>17 (22.4%)</td>
<td>3 (4.5%)</td>
<td>8.046</td>
<td>0.002*</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>14 (18.4%)</td>
<td>14 (20.9%)</td>
<td>0.138</td>
<td>0.710</td>
</tr>
<tr>
<td>Coughing at night</td>
<td>36 (47.4%)</td>
<td>20 (29.9%)</td>
<td>4.586</td>
<td>0.032*</td>
</tr>
<tr>
<td>Fever</td>
<td>16 (21.1%)</td>
<td>25 (37.3%)</td>
<td>4.604</td>
<td>0.032*</td>
</tr>
<tr>
<td>Wheezing</td>
<td>22 (28.9%)</td>
<td>22 (32.8%)</td>
<td>0.253</td>
<td>0.615</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>11 (14.5%)</td>
<td>10 (14.9%)</td>
<td>0.006</td>
<td>0.939</td>
</tr>
<tr>
<td>Wet rales</td>
<td>36 (47.4%)</td>
<td>45 (67.2%)</td>
<td>5.682</td>
<td>0.017*</td>
</tr>
<tr>
<td>Wheezes</td>
<td>18 (23.7%)</td>
<td>18 (26.9%)</td>
<td>0.191</td>
<td>0.662</td>
</tr>
<tr>
<td>No rales</td>
<td>13 (17.1%)</td>
<td>13 (19.4%)</td>
<td>0.369</td>
<td>0.544</td>
</tr>
<tr>
<td>Usage of macrolides</td>
<td>63 (82.9%)</td>
<td>51 (76%)</td>
<td>1.011</td>
<td>0.315</td>
</tr>
<tr>
<td>Total disease duration (days)</td>
<td>28.3±2.1</td>
<td>24.3±2.3</td>
<td>t=1.717</td>
<td>0.087</td>
</tr>
</tbody>
</table>

BP: Bordetella pertussis. *: P < 0.05.

Table 5. Comparison of cell proportion and image changes between the BP group and non-BP group

<table>
<thead>
<tr>
<th>Feature</th>
<th>BP group (n=76)</th>
<th>Non-BP group (n=67)</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell count &gt; 15×10^9/L</td>
<td>28 (36.8%)</td>
<td>9 (13.4%)</td>
<td>10.174</td>
<td>0.001*</td>
</tr>
<tr>
<td>Percent of lymphocytosis &gt; 60%</td>
<td>53 (69.7%)</td>
<td>34 (50.7%)</td>
<td>5.390</td>
<td>0.020*</td>
</tr>
<tr>
<td>No abnormality in image results</td>
<td>27 (35.5%)</td>
<td>15 (22.4%)</td>
<td>2.963</td>
<td>0.085</td>
</tr>
<tr>
<td>Pneumonia detected in image results</td>
<td>49 (64.5%)</td>
<td>47 (70.1%)</td>
<td>0.732</td>
<td>0.392</td>
</tr>
</tbody>
</table>

BP: Bordetella pertussis. *: P < 0.05.
lymphocytes may suggest the diagnosis of pertussis [13, 34]. These results may be related to the choice of the chosen subjects. In the current study, cases in the BP group and non-BP group had onset in all four seasons. No statistical differences were found between the groups. The BP group had a higher incidence of the disease in the spring, summer, and autumn. This result was not consistent with previous investigations which revealed that pertussis had higher incidence rates in the spring and summer. Present results indicated that prevalence of BP had different seasonal distribution characteristics depending on geographical changes. It may also be related to the mutation of BP strains, requiring further study. Effective treatment using macrolide antibiotics in the early period (before the appearance of paroxysmal coughing) for BP infections can reduce the severity of coughing and shorten the duration. If treatment is performed after the early period, the therapeutic effects may be limited [35]. Therefore, it is particularly important to improve etiological diagnosis methods of pertussis-like syndrome in early clinical stages.

Conclusion

In the current study, bacterial culturing and qRT-PCR methods were used to detect pathogens in the NPA of 280 children with pertussis syndrome in Hunan Province. The aim of the current study was to examine the pathogenic spectrum and clinical characteristics of children with pertussis syndrome. Results of the current study may provide a scientific basis for clinical empirical treatment and may provide reference for the adjustment of health prevention and control policies.

Acknowledgements

This study was supported by the Hunan Provincial Natural Science Foundation (No. 2017-JJ2150).

Disclosure of conflict of interest

None.

Address correspondence to: Lili Zhong, Children’s Medical Center, Hunan Provincial People’s Hospital, No. 61, Jie-Fang Road, Changsha 410005, Hunan Province, P. R. China. Tel: +86-0731-83929419; E-mail: 570047414@qq.com

References

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