Int J Clin Exp Med 2019;12(8):10769-10775
www.ijcem.com /ISSN:1940-5901/IJCEM0091270

Original Article

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

Xiaojuan Lin[1], Lili Zhong[1], Yujiao Luo[1], Zhongping Deng[2], Liangyi Xie[1], Fang Peng[1], Han Huang[1], Liping Liu[1], Min Chen[1], Danxia Peng[1], Xiaofang Ding[1], Manzhi Wang[3], Haiying Han[4], Ge Zhou[5], Yuhui Huang[6], Ping Jiang[7]

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

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 concussions 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, χ^2 tests, continuous correction χ^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-

Table 1. Distribution characteristics of positive detected pathogens (N=280)

Pathogen types	Positive samples number	Detection rate
BP	135	48.2%
RSV	29	10.4%
MP	24	8.6%
HPIV3	23	8.2%
ADV	20	7.1%
Kp	11	3.9%
FluA	10	3.6%
HBoV	8	2.9%
RHV	8	2.9%
HI	8	2.9%
E.coli	7	2.5%
Streptococcus pneumoniae	5	1.8%
CP	3	1.1%
HCoV-OC43	3	1.1%
Pseudomonas aeruginosa	3	1.1%
FluB	2	0.7%
HCoV-229E	2	0.7%
Moraxelle catarrhalis	2	0.7%
Acinetobacter baumannii	2	0.7%
Burkholderia cepacia	1	0.4%
Enterobacter aerogenes	1	0.4%
HPIV2	1	0.4%

BP: *Bordetella pertussis*; RSV: *Respiratory syncytial virus*; MP: *Mycoplasma Pneumoniae*; HPIV3: *Human parainfluenza virus 3*; ADV: *Adenovirus*; Kp: *Klebsiella pneumoniae*; FluA: *influenza A*; HBoV: *Human bocavirus*; RHV: *rhinovirus*; HI: *Hemophilus influenza*; E. coli: *Escherichia coli*. Streptococcus pneumoniae; CP: chlamydia pneumoniae; HCoV-OC43: *human coronavirus OC43*; FluB: *influenza B*; HCoV-229E: *human coronavirus 229E*; HPIV2: *human parainfluenza virus 2*.

gens 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 infection 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 (χ^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 (χ^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 (χ^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-

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

	BP group (n=76)	Non-BP group (n=67)	χ^2	*P* value
Male	39 (51.3%)	41 (61.2%)	1.41	0.235
Age (≤12 months)	71 (93.4%)	53 (79.1%)	6.335	0.012*
Vaccination rate	40 (52.6%)	33 (49.3%)	1.032	0.310
Exposure history of family members of respiratory tract infections within 21 days	20 (26.3%)	10 (14.9%)	2.787	0.095

BP: *Bordetella pertussis*. *: $P < 0.05$.

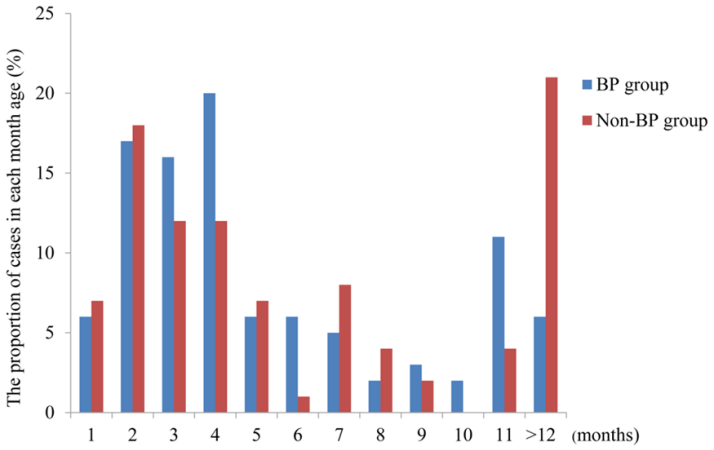

Figure 1. Proportion of cases in each month age. The figure shows the proportion of cases of the BP group and non-BP group in each month of age. BP: *Bordetella pertussis*.

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

	BP group (n=76)	Non-BP group (n=67)	χ^2	*P* value
Spring	17 (22.4%)	10 (14.9%)	1.288	0.256
Summer	20 (26.3%)	23 (34.3%)	1.087	0.297
Autumn	31 (40.8%)	23 (34.3%)	0.632	0.426
Winter	8 (10.5%)	11 (16.5%)	1.073	0.300

BP: *Bordetella pertussis*.

toms for cases in the BP group and non-BP group are shown in **Table 4**. 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. Most of the children in the two groups were treated with macrolides, with a dose of 20-30 mg/kg/d. Usage rates of macrolides were 82.9% (63/76) and 76.1% (51/67) in BP and non-BP groups, respectively. No significant differences were found (χ^2=1.011, P=0.315).

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

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].

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

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

BP: *Bordetella pertussis*. *: $P < 0.05$.

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

	BP group (n=76)	Non-BP group (n=67)	χ^2	P
White blood cell count > 15×10^9/L	28 (36.8%)	9 (13.4%)	10.174	0.001*
Percent of lymphocytosis > 60%	53 (69.7%)	34 (50.7%)	5.390	0.020*
No abnormality in image results	27 (35.5%)	15 (22.4%)	2.963	0.085
Pneumonia detected in image results	49 (64.55%)	47 (70.1%)	0.732	0.392

BP: *Bordetella pertussis*. *: $P < 0.05$.

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, ADV, 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

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

[1] Hozbor D, Mooi F, Flores D, Weltman G, Bottero D, Fossati S, Lara C, Gaillard ME, Pianciola L, Zurita E, Fioriti A, Archuby D, Galas M, Binsztein N, Regueira M, Castuma C, Fingermann M, Graieb A. Pertussis epidemiology in Argentina: trends over 2004-2007. J Infect 2009; 59: 225-231.

[2] Ridda I, Yin JK, King C, Raina MacIntyre C and McIntyre P. The importance of pertussis in older adults: a growing case for reviewing vaccination strategy in the elderly. Vaccine 2012; 30: 6745-6752.

[3] Wang L, Lei D and Zhang S. Acellular pertussis vaccines in China. Vaccine 2012; 30: 7174-7178.

[4] Lu CY, Tsai HC, Huang YC, Huang DT, Liu CC, Huang LM; TPIDA. A national seroepidemiologic survey of pertussis among school children in Taiwan. Pediatr Infect Dis J 2017; 36: e307-e312.

[5] Seib K, Pollard AJ, De WP, Andrews RM, Zhou F, Hatchett RJ, Pickering LK and Orenstein WA. Policy making for vaccine use as a driver of vaccine innovation and development in the developed world. Vaccine 2017; 35: 1380-1389.

[6] Cherry JD. Epidemic pertussis in 2012--the resurgence of a vaccine-preventable disease. N Engl J Med 2012; 367: 785-787.

[7] Liu XC, Bell CA, Simmonds KA, Svenson LW, Fathima S, Drews SJ, Schopflocher DP and Russell ML. Epidemiology of pertussis in Alberta, Canada 2004-2015. Bmc Public Health 2017; 17: 539.

[8] Goldstein ND, Newbern EC, Tabb LP and Welles SL. Density of day cares in relation to reported pertussis incidence in Philadelphia. Public Health 2017; 146: 126-133.

[9] Sadkowska-Todys M, Zieliński A and Czarkowski MPMP. Infectious diseases in Poland in 2015. Przegl Epidemiol 2017; 71: 295-309.

[10] Ferrer A, Calicó I, Manresa JM, Andreu A, Moraga F and Valle I. [Microorganisms isolated in cases of pertussis-like syndrome]. Enferm Infecc Microbiol Clin 2000; 18: 433-438.

[11] Lotfi MN, Nikbin VS, Nasiri O, Badmasti F and Shahcheraghi F. Molecular detection of bordetella holmesii in two infants with pertussis-like syndrome: the first report from Iran. Iran J Microbiol 2017; 9: 219-223.

[12] Aquino-Andrade A, Martínez-Leyva G, Mérida-Vieyra J, Saltigeral P, Lara A, Domínguez W, García de la Puente S, De Colsa A. Real-time polymerase chain reaction-based detection of bordetella pertussis in Mexican infants and Their Contacts: a 3-year multicenter study. J Pediatr 2017; 188: 217-223.

[13] Phadke VK, Mccracken JP, Kriss JL, Lopez MR, Lindblade KA, Bryan JP, Garcia ME, Funes CE and Omer SB. Clinical characteristics of hospitalized infants with laboratory-confirmed pertussis in Guatemala. J Pediatric Infect Dis Soc 2017; 7: 310-316.
[14] Nikbin VS, Shahcheraghi F, Lotfi MN, Zahraei SM and Parzadeh M. Comparison of culture and real-time PCR for detection of bordetella pertussis isolated from patients in Iran. Iran J Microbiol 2013; 5: 209-214.
[15] Vabret A, Mouthon F, Mourez T, Gouarin S, Petitjean J and Freymuth F. Direct diagnosis of human respiratory coronaviruses 229E and OC43 by the polymerase chain reaction. J Virol Methods 2001; 97: 59-66.
[16] Pham TT, Burchette JL Jr, Hale LP. Fatal disseminated adenovirus infections in immunocompromised patients. Am J Clin Pathol 2003; 120: 575-583.
[17] Lu X, Chittaganpitch M, Olsen SJ, Mackay IM, Sloots TP, Fry AM and Erdman DD. Real-time PCR assays for detection of bocavirus in human specimens. J Clin Microbiol 2006; 44: 3231-3235.
[18] Faux CE, Arden KE, Lambert SB, Nissen MD, Nolan TM, Chang AB, Sloots TP and Mackay IM. Usefulness of published PCR primers in detecting human rhinovirus infection. Emerg Infect Dis 2011; 17: 296-298.
[19] Lee HK, Lee CK, Loh TP, Tang JW, Chiu L, Tambyah PA, Sethi SK, Koay ES. Diagnostic testing for pandemic influenza in Singapore: a novel dual-gene quantitative real-time RT-PCR for the detection of influenza A/H1N1/2009. J Mol Diagn 2010; 12: 636-643.
[20] Boivin G, Côté S, Déry P, De Serres G and Bergeron MG. Multiplex real-time PCR assay for detection of influenza and human respiratory syncytial viruses. J Clin Microbiol 2004; 42: 45-51.
[21] Huijskens EG, Biesmans RC, Buiting AG, Obihara CC and Rossen JW. Diagnostic value of respiratory virus detection in symptomatic children using real-time PCR. Virol J 2012; 9: 276.
[22] Apfalter P, Assadian O, Blasi F, Boman J, Gaydos CA, Kundi M, Makristathis A, Nehr M, Rotter ML and Hirschl AM. Reliability of nested PCR for detection of chlamydia pneumoniae DNA in atheromas: results from a multicenter study applying standardized protocols. J Clin Microbiol 2002; 40: 4428-4434.
[23] Chang HY, Chang LY, Shao PL, Lee PI, Chen JM, Lee CY, Lu CY, Huang LM. Comparison of real-time polymerase chain reaction and serological tests for the confirmation of Mycoplasma pneumoniae infection in children with clinical diagnosis of atypical pneumonia. J Microbiol Immunol Infect 2014; 47: 137-144.
[24] Mattoo S and Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to bordetella pertussis and other bordetella subspecies. Clin Microbiol Rev 2005; 18: 326-382.
[25] Liu X, Wang Z, Zhang J, Li F, Luan Y, Li H, Li Y and He Q. Pertussis outbreak in a primary school in China: infection and transmission of the macrolide-resistant bordetella pertussis. Pediatr Infect Dis J 2017; 37: e145-e148
[26] Alamaw SD, Kassa AW and Gelaw YA. Pertussis outbreak investigation of Mekdela district, South Wollo zone, Amhara region, North-West Ethiopia. BMC Res Notes 2017; 10: 420.
[27] Sarbay H, Polat A, Mete E, Balci YI and Akin M. Pertussis-like syndrome associated with adenovirus presenting with hyperleukocytosis: case report. North Clin Istanb 2016; 3: 140-142.
[28] Zouari A, Smaoui H, Njamkepo E, Mnif K, Ben Jaballah N, Bousnina S, Barsaoui S, Sammoud A, Ben Becher S, Guiso N, Kechrid A. [The re-emergence of pertussis in Tunisia]. Med Mal Infect 2011; 41: 97-101.
[29] Huang H, Deng L, Xiao F, Fu J, Deng J, Wang F and Yang J. [Clinical analysis of children with pertussis and significance of respiratory virus detection in the combined diagnosis]. Zhonghua Er Ke Za Zhi 2017; 55: 580-585.
[30] Frassanito A, Nenna R, Nicolai A, Pierangeli A, Tozzi AE, Stefanelli P, Carsetti R, Concato C, Schiavoni I and Midulla F; Pertussis study group. Infants hospitalized for Bordetella pertussis infection commonly have respiratory viral coinfections. BMC Infect Dis 2017; 17: 492.
[31] Jõgi P, Oona M, Kaart T, Toompere K, Maskina T, Koort I, Rätsep A and Lutsar I. Pertussis and parapertussis in children and adults with a persistent cough: an observational study. Infection 2018; 46: 83-91.
[32] Gökçe Ş, Kurugöl Z, Aydemir SŞ, Çiçek C, Aslan A and Koturoğlu G. Bordetella pertussis infection in hospitalized infants with acute bronchiolitis. Indian J Pediatr 2018; 85: 189-193.
[33] Al Maani A, Al Qayoudhi A, Nazir HF, Omar H, Al Jardani A, Al Muharrmi Z and Wali Y. Pertussis and pertussis like illness: pediatric experience in Oman. Oman Med J 2017; 32: 396-402.
[34] Levene I and Wacogne I. Question 3. is measurement of the lymphocyte count useful in the investigation of suspected pertussis in infants? Arch Dis Child 2011; 96: 1203-1205.
[35] Tiwari T, Murphy TV and Moran J; National Immunization Program, CDC. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC guidelines. MMWR Recomm Rep 2005; 54: 1-16.