Original Article

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Clinical Utility of Molecular Diagnostics in Children with Respiratory Infections

Çocuklarda Solunum Yolu Enfeksiyonlarında Moleküler Tanı Yöntemlerinin Klinikte Kullanımı

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ABSTRACT

Objective: Lower respiratory tract infections (LRTI) are among the major causes of mortality in children worldwide. Our study aimed to determine viral agents in children under five years of age who were followed up with acute LRTI.

Methods: Nasopharyngeal swab samples were taken from children aged 1 month to 5 years who were diagnosed with LRTI at the Bezmialem Vakıf University pediatric emergency department between March 1, 2015 and January 31, 2016. Cases with underlying chronic diseases were excluded from the study. The presence of the agent was investigated in the samples taken using the real-time polymerase chain reaction method. The distribution of cases in which the agent was detected according to age groups and seasons was examined. The relationship between the agent and the clinical and laboratory findings was investigated.

Results: Of the 95 patients included in the study, 51 (53.7%) were male, 44 (46.3%) were female, and the mean age was 26.2 months. The presence of viruses was shown in 50 cases (52.6%). The most frequently detected viruses were rhinovirus (28%), human bocavirus (HBoV) (26%) and respiratory syncytial virus (RSV) (24%). While RSV infections were more common in winter months, HBoV was observed to persist throughout the year. No significant relationship was found between the clinical and laboratory findings of the patients and the agents.

Conclusion: Viral etiology was detected in 52.6% of the cases. Molecular tests are valuable in detecting more than one virus in the distribution of viruses among different age groups.

Keywords: Infections, respiratory tract, multiplex polymerase chain reaction

ÖZ

Amaç: Alt solunum yolu enfeksiyonları (ASYE) tüm dünyada çocuklarda mortalite nedenleri arasında önemli yer tutmaktadır. Çalışmamızda akut ASYE tanısıyla izlenen beş yaş altındaki çocuklarda viral etkenlerin belirlenmesi amaçlanmıştır.

Yöntemler: Bezmialem Vakıf Üniversitesi çocuk acil servisinde 1 Mart 2015-31 Ocak 2016 tarihleri arasında ASYE tanısı alan 1 ay-5 yaş arası çocuklardan nazofarengeal sürüntü örnekleri alınmıştır. Altta yatan kronik hastalığı olan olgular çalışma dışı bırakılmıştır. Alınan örneklerde gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle etken varlığı araştırılmıştır. Etken tespit edilen olguların yaş gruplarına ve mevsimlere göre dağılımı incelenmiştir. Klinik ve laboratuvar bulguları ile etken ilişkisi araştırılmıştır.

Bulgular: Çalışmaya dahil edilen 95 hastanın 51'i (%53,7) erkek, 44'ü (%46,3) kız olup yaş ortalaması 26,2 aydır. Virüs varlığı 50 olguda (%52,6) gösterilmiştir. En sık saptanan virüsler, rinovirüs (%28), insan bocavirüs (HBoV) (%26) ve respiratuvar sinsiyal virüstür (RSV) (%24). RSV enfeksiyonları daha çok kış aylarında görülürken HBoV'nin yıl boyu sürdüğü gözlenmiştir. Hastaların klinik ve laboratuvar bulguları ile ajanlar arasında anlamlı ilişki saptanamamıştır.

Sonuç: Olguların %52,6'sında viral etiyoloji saptanmıştır. Moleküler testler virüslerin farklı yaş grupları arasındaki dağılımında birden fazla virüsün tespitinde değerlidir.

Anahtar Sözcükler: Enfeksiyonlar, solunum yolu, multipleks polimeraz zincir reaksiyonu

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Introduction

Lower respiratory tract infection (LRTI) is one of the most common conditions encountered in pediatric emergency departments during the winter months. Viruses are the most common etiological agents of LRTIs in infants and children under 5 years of age (1,2). Respiratory syncytial virus (RSV) is responsible for more than 50% of bronchiolitis cases (3). RSV and influenza viruses have been reported to cause 66,000-111,000 deaths annually in developing countries (3,4). In Türkiye, LRTI is the 6th most common disease exposed by children 0-6 years age (5).

Knowledge of the viral etiology may help cohorting in hospitalized patients or deciding on antiviral therapy. Although routine use of rapid diagnostic tests during emergency department is not recommended, it may decrease antibiotic use (1). In a retrospective cohort study among hospitalized children, multiplex polymerase chain reaction (PCR) tests for identification of respiratory viruses led to a decrease in utilization of healthcare resource (6).

Studies investigating viral pathogens in children with LRTI are limited in Türkiye (7-15). In this study, we sought to determine the predominant viral etiologies of LRTI in children at a tertiary care center in İstanbul. We also searched viral-viral detections and associations with the clinical findings.

Methods

Study Setting

Bezmialem Vakıf University Hospital is one of the sentinel hospitals in İstanbul. During the study period of March 2015 and January 2016, 271,625 children were treated at the emergency department.

Sample Detection

One hundred children attending to emergency department between March 2015 and January 2016 were tested with PCR methods for respiratory pathogens. Informed consent was requested to enter the study.

A nasopharyngeal sample was obtained from patients aged between 1 month and 5 years who had diagnosis of bronchitis, bronchiolitis, and pneumonia characterized by acute-onset cough or difficulty in breathing with fast breathing for age. Consolidation, infiltrate or effusion on chest X-ray (CXR) were considered as radiographic evidences of pneumonia (1). Patients with underlying diseases including cystic fibrosis, congenital heart disease, recurrent wheezing, neuromuscular diseases, and immunodeficiency; and those who had been hospitalized during the last 14 days were excluded. Data including age, sex, symptoms and signs at presentation, blood leukocyte count, C-reactive protein (CRP), CXR findings were recorded. Seventeen viruses: RSV; influenza A and B viruses; human parainfluenza virus (HPIV) types 1-4; human adenovirus (HAdV); human rhinovirus (HRV); human metapneumovirus (HMPV); human coronavirus (HCoV) (NL63, 229E, OC43, HKU1), human bocavirus (HBoV), human enterovirus (EV), and parechovirus (PeV); and five bacteria: *Chlamydia pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* type B, *Staphylococcus aureus,* and *Mycoplasma pneumonia*) were investigated with real-time PCR (RT-PCR). Among bacteria, only *C. pneumoniae* and *M. pneumoniae* were accepted as pathogens. The samples which were taken from the patient's nose by nasopharyngeal swab with 2-3 rotations were sent to the microbiology lab within maximum 30 minutes. After extraction of clinical samples, a commercial RT-PCR kit (FTD respiratory 21 (Fast Track) was used for identification of DNA and RNA of the respiratory pathogens. This kit used a set including primers and TaqMan probes which can detect one or more of these viruses. When the viral nucleic acid was of an RNA virus, a reverse transcription process occurred for production of cDNA. Then cDNA was duplicated by PCR using a primary probe specific to that virus. During PCR, amplicons were detected by measuring fluorescent radiation. None of the patients needed hospital treatment.

This study was approved by Bezmialem Vakıf University Clinical Research Ethics Committee with decision no 610/21 (date: 04.06.2014). The study was funded by Bezmialem Vakıf University (project number: 18245212-108.99).

Statistical Analysis

The SPSS 15.0 statistical program was used for analysis of the data. Demographic and clinical characteristics of patients with no virus detection, virus positive patients, and patients with viral-viral co-detection were compared using the Pearson chi-square and Kruskal-Wallis tests according to variable type.

Results

Patient Characteristics

Among 100 patients evaluated five were excluded because of underlying comorbidities. Among 95 children, mean age was 26.2 months and 53.7% were male (Table 1). Cough, wheezing, and fever were the most common clinical findings (100%, 70%, and 60% respectively). Mean leukocyte count was 11,600/mm^3 and CRP level was 2.84 mg/dL. Radiographic findings were hyperinflation, interstitial infiltrations, lineary atelectasis, lobar consolidation, peribronchial thickening, and lobar atelectasis. None were hospitalized or needed intensive care management.

Respiratory Viruses

Sixty two respiratory viruses from 50 patients were detected. The most commonly detected viruses were human rhinovirus (28%) followed by hBoV (26%), and RSV (24%) (Table 2). The mean age of children with human rhinovirus, HBoV and RSV infections were 25.7 months, 26.7 months, and 24.7 months, respectively. Co-detection of ≥2 viral pathogens was found in 11 patients (Table 3). The most common viral co-detection was HBoV and HMPV (3 patients). While the majority (72.7%) of HBoV infections presented as viral co-infections, all samples with HPIV positivity were single infections. RSV infections were mostly seen in the winter. However, HBoV was found throughout the year (Figure 1).

Table 1. Demographic and clinical characteristics of pediatric patients with LRTI

Variable (%)	No virus detected (n=45)	Single virus positive (n=39)	Viral-viral coinfection (n=11)	p-value
Age (months)[a]	28.06	21.79	35.09	0.107
Symptoms (%)				
Wheezing	73.3	64.1	81.8	0.445
Fever	51.1	64.1	81.8	0.140
Nasal discharge	44.4	38.5	45.5	0.834
Difficulty in breathing	20	17.9	27.3	0.792
Nasal obstruction	6.7	7.7	0	0.645
Cyanosis	0	2.6	9.1	0.164
Signs (%)				
Rales	60	71.8	72.7	0.465
Prolonged expirium	48.9	69.2	54.5	0.165
Rhonchi	42.2	25.6	63.6	0.051
Retractions	28.9	30.8	18.2	0.713
Lab findings				
WBC	11.545	12.422	12.100	0.577
PNL (%)	50.52	50.05	57.90	0.540
CRP	1.71	2.30	1.56	0.581
CXR ordered (%)	80	89.7	100	0.161
Use of antibiotics (%)	36.4	53.8	50	0.265

[a]Mean, WBC: White blood cells, PNL: Polymorphonuclear leukocytes, CRP: C-reactive protein, CXR: Chest X-ray, LRTI: Lower respiratory tract infection

Table 2. Distribution of viruses according to age groups

	Total n (%)	Single infection n (%)	Viral-viral co-infection n (%)	Mean age, months (SD)	Age (months)			
					1-6	7-12	13-24	25-60
HRV	14 (28)	13 (92.8)	1 (7.1)	25.7 (17.1)	1 (7.1)	4 (28.5)	2 (14.2)	7 (50)
HBoV	13 (26)	5 (38.4)	8 (61.5)	26.7 (20.1)	2 (15.3)	4 (30.7)	1 (7.6)	6 (46.1)
RSV	12 (24)	7 (58.3)	5 (41.6)	24.7 (18.2)	1 (12.5)	4 (23.5)	2 (28.5)	5 (16.6)
HPIV	8 (16)	8 (100)	0	26.8 (22.7)	3 (37.5)	1 (5.8)	0	4 (13.3)
HMPV	4 (8)	1 (25)	3 (75)	33 (12.3)	0	2 (11.7)	0	2 (6.6)
HAdV	3 (6)	1 (33.3)	2 (66.6)	48 (12)	0	0	0	3 (10)
PeV	3 (6)	2 (66.6)	1 (33.3)	10.8 (6.3)	1 (12.5)	0	2 (28.5)	0
HCoV	3 (6)	2 (66.6)	1 (33.3)	27 (15.5)	0	1 (5.8)	0	2 (6.6)
Human EV	2 (4)	0	2 (100)	24 (16.9)	0	1 (5.8)	0	1 (3.3)
Total	50	39 (78)	11 (22)	26.3 (20.7)	8 (16)	15 (30)	5 (10)	22 (44)

SD: Standard deviation, HRV: Human rhinovirus, HBoV: Human bocavirus, RSV: Respiratory syncytial virus, HPIV: Human parainfluenza virus, HMPV: Human metapneumovirus, HAdV: Human adenovirus, PeV: Parechovirus, HCoV: Human coronavirus, EV: Enterovirus

Comparison of Patients with No Virus Detection, Single Virus Positive Patients, and Patients with Viral-viral Co-detection

Mean age of subjects with viral-viral co detection was higher than those with single infections (35 months vs 21.7 months) but without reaching statistical significance (Table 1). Clinical findings, order of chest radiographs, and use of antibiotics were not significantly different between the three groups.

Viral-bacterial Co-detection

Twenty one patients had simultaneous detection of bacteria with viruses. *M. pneumoniae* (1 of 5), *H. influenzae* type b (5 of 7), *S. aureus* (4 of 8), and *S. pneumoniae* (19/28) were detected as co-detections with viruses.

Table 3. Distribution of viruses in patients with viral-viral co-infection

Pathogens detected	Number (%)	Mean age, months
Co-infection, two viruses	10	
HBoV+HMPV	3 (27.2)	40
RSV+HboV	2 (18.1)	36
HBoV+HAdV	1 (9)	36
HBoV+human EV	1 (9)	12
RSV+PeV	1 (9)	14
RSV+HAdV	1 (9)	60
HRV+human EV	1 (9)	36
Co-infection, three viruses	1	
RSV+HBoV+HCoV-NL63	1 (9)	36
Total	11 (100)	35

HBoV: Human bocavirus, HMPV: Human metapneumovirus, RSV: Respiratory syncytial virus, HAdV: Human adenovirus, EV: Enterovirus, PeV: Parechovirus, HRV: Human rhinovirus, HCoV: Human coronavirus

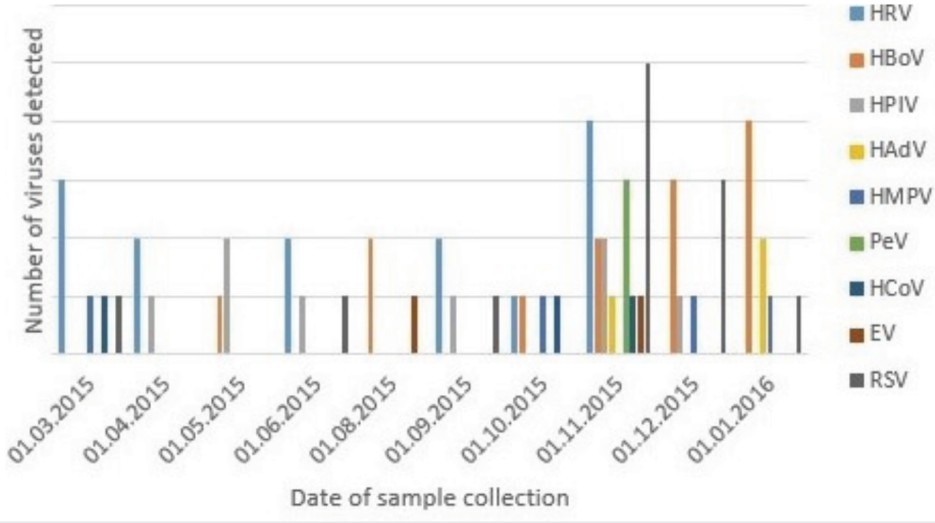

Figure 1. Comparison of viral seasonality

HBoV: Human bocavirus, HMPV: Human metapneumovirus, RSV: Respiratory syncytial virus, HAdV: Human adenovirus, EV: Enterovirus, PeV: Parechovirus, HRV: Human rhinovirus, HCoV: Human coronavirus

Discussion

Here we investigated a plenty of respiratory pathogens (17 viruses) among children with LRTI. Viruses were detected in 52.6% of patients. HRV (28%), HBoV (26%), and RSV (24%) were the most commonly identified pathogens. Other detected viruses were HPIV (16%), HMPV (8%), HAdV (6%), PeV (6%), HCoV (6%), and human EV (4%). RSV infections were mostly seen in the winter. However, HBoV infections were found throughout the year. Adenoviral infections occurred in older children, and PeVs were detected in younger children (mean age 48 months vs 10.8 months, respectively. Among infants and young children with bronchiolitis and pneumonia, RSV is the most common etiological agent which can lead to epidemics in winter and early spring. An observational and prospective study in Spain identified RSV in 52.9% of children with LRTI (16). A previous study from Türkiye identified RSV as the leading pathogen (55.6%) followed by HPIV (27.8%), HMPV (13%), influenza A virus (9.3%), and HAdV (5.6%) (7). Gökçe et al. (8) examined 316 children younger than 24 months who were hospitalized for acute viral bronchiolitis. Of the 316 children, 237 showed at least one respiratory tract pathogen (75% of the participants). RSV was the most common virus (40.1%) followed by HRV (n=78, 24.6%). HRV, the etiologic agent of common cold, may contribute to community acquired pneumonia. It is one of the most commonly detected respiratory viruses among childhood respiratory diseases (12-79%) (17). Since it is frequently identified as a co-infecting pathogen and among asymptomatic children, the role of HRV in severe LRTI is unclear (18). HBoV infection prevelance was reported as

5-19% in children with acute LRTI (19). Midilli et al. (14) reported that HBoV was identified in 6.5% of children with LRTI. Uyar et al. (13) evaluated 62 children with bronchiolitis in comparison to 33 healthy children for the presence of respiratory viruses. HBoV was detected in 4.8% of patients. HRV was the most commonly detected virus in the control group while none of the samples was positive for HBoV (13). Demirci et al. (15) showed HBoV positivity rate as 6.7% for children 1 month to 56 months. Having of a sick sibling and number of children at home increased the risk (15).

We identified that 10.5% patients had two or more respiratory viruses. Among co-detected viruses HBoV was the leading virus. HBoV is reported as an infrequent sole cause of acute bronchiolitis (20). Children with viral co-detections tended to be older than those with single infections. Co-infection with multiple viruses has been reported in 8.4-36.1% of children with LRTI (21). Cebey-López et al. (16) conducted an observational, prospective study in Spain among children with LRTI and showed that 45.1% of children had viral co-infections (11). The most frequent co-infection patterns were RSV-HRV. Among a comparison cohort of 97 children from United Kingdom, 5 patients (5.2%) had viral co-infections (RSV-HBoV/HBoV-influenza). Gökçe et al. (8) performed a cross-sectional, descriptive study in İzmir, with 316 children younger than 24 months who were hospitalized for acute viral bronchiolitis. RSV and HRV had the highest co-infection rate (22.3%).

There are conflicting data on whether viral co-infection is associated with increased severity in children with respiratory infections (21-23). We did not find any significant differences in clinical or laboratory findings, order of chest radiographs, and use of antibiotics among patients with no virus detection, virus positive patients, and patients with viral co-detection. There is substantial diversity in the management of LRTI reflecting the absence of a consensual treatment. Although combinations of clinical features have been proposed to distinguish viral from bacterial diseases, none are sufficiently sensitive to be reliable or widely used. Subramony et al. (6) reported that identification of a viral etiological agent decreased use of antibiotics and chest radiographs among children hospitalized.

Viruses and bacteria residing in the upper respiratory tract have complex interactions. In this study *S. pneumoniae* and *H. influenzae* were more likely to be present in the nasopharynx in combination with respiratory viruses.

Study Limitations

A limitation of our study was that there was no control group. Many types of bacteria and viruses can be present as part of the natural flora without causing an infection. A systematic literature review of 23 studies showed that RSV, influenza viruses, parainfluenza viruses, and HMPV had strong evidence for LRTI (24). HRV was only weakly associated with LRTI symptoms.

The second limitation was that patients were enrolled during only 11 months. The cost of broadly multiplexed molecular test is high and only 100 patients could be selected for sampling. Surprisingly, there were no influenza positive cases. According to Turkish Ministry of Health General Directorate of Public Health, influenza activity in Türkiye started at October, reached peak at December, and finished at the end of February in 2015. In 2016, influenza cases cumulated between February and March. Since the study was completed at January we could have missed influenza cases. Long term follow up is needed to obtain more important information.

Conclusion

We detected viruses in more than half of children with LRTI. Although some of these viruses might represent carriage we thought that at least one third were true pathogens. There was no significant difference in clinical findings or clinical course between patients with no virus detection, with single virus detection, and with double/multiple detection. Interestingly, respiratory symptoms such as wheezing and fever were slightly higher in the "coinfection" group and there was a higher rate of antimicrobial prescriptions (36.4-53.8%) in patients with identified viruses. Molecular tests are helpful to identify the etiology of LRTI. However the efficacy was limited in the emergency department setting unless results were obtained quickly and clinicians were notified.

Ethics

Ethics Committee Approval: This study was approved by Bezmialem Vakıf University Clinical Research Ethics Committee with decision no 610/21 (date: 04.06.2014).

Informed Consent: Informed consent was requested to enter the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Concept: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Design: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Data Collection or Processing: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Analysis or Interpretation: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Literature Search: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T., Writing: İ.T., B.S., B.B.D., U.E., F.U.K., Ö.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Harris M, Clark J, Coote N, Fletcher P, Harnden A, McKean M, et al. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax. 2011;66(Suppl 2):1-23.
2. Friedman JN, Rieder MJ, Walton JM; Canadian Paediatric Society, Acute Care Committee, Drug Therapy and Hazardous Substances Committee. Bronchiolitis: Recommendations for diagnosis,

monitoring and management of children one to 24 months of age. Paediatr Child Health. 2014;19:485-91.

3. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. 2010;375:1545-55.
4. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet. 2011;378:1917-30.
5. Turkey in Statistics 2015. TurkStat, Turkey Health Interview Survey, 2014. https://ec.europa.eu/eurostat/documents/7330775/7339623/Turkey+_in_statistics_2015.pdf/317c6386-e51c-45de-85b0-ff671e3760f8
6. Subramony A, Zachariah P, Krones A, Whittier S, Saiman L. Impact of Multiplex Polymerase Chain Reaction Testing for Respiratory Pathogens on Healthcare Resource Utilization for Pediatric Inpatients. J Pediatr. 2016;173:196-201.
7. Hatipoğlu N, Somer A, Badur S, Unüvar E, Akçay-Ciblak M, Yekeler E, et al. Viral etiology in hospitalized children with acute lower respiratory tract infection. Turk J Pediatr. 2011;53:508-16.
8. Gökçe Ş, Kurugöl Z, Koturoğlu G, Çiçek C, Aslan A. Etiology, Seasonality, and Clinical Features of Viral Respiratory Tract Infections in Children Hospitalized With Acute Bronchiolitis: A Single-Center Study. Glob Pediatr Health. 2017;4: 2333794X17714378.
9. Bicer S, Giray T, Çöl D, Erdağ GÇ, Vitrinel A, Gürol Y, et al. Virological and clinical characterizations of respiratory infections in hospitalized children. Ital J Pediatr. 2013;39:22.
10. Akçalı S, Yılmaz N, Güler O, Şanlidağ T, Anıl M. The frequency of respiratory tract viral agents in children with lower respiratory tract infections. Turk Arch Ped. 2013;48:215-20.
11. Sancaklı O, Yenigun A, Kirdar S. Results of Polymerase Chain Reaction in Nasopharyngeal Swab Specimens of Patients with Lower Respiratory Tract Infection. J Pediatr Inf. 2012;6:84-9.
12. Yuksel H, Yilmaz Ö, Akcali S, Sögüt A, Ciftdogan Yilmaz D, Urk V, et al. Common viral etiologies of community acquired lower respiratory tract infectıons in young children and their relationship with long term complicatıons. Mikrobiyoloji Bul. 2008;42:429-35.
13. Uyar M, Kuyucu N, Tezcan S, Aslan G, Taşdelen B. Determination of the frequency of Human Bocavirus and other respiratory viruses among 0-2 years age group children diagnosed as acute bronchiolitis. Microbiol Bul. 2014;48:242-58.
14. Midilli K, Yılmaz G, Türkoğlu S, Iskanova B, Ergin S, Yarımcam F, et al. Detection of human bocavirus DNA by polymerase chain reaction in children and adults with acute respiratory tract infections. Microbiol Bul. 2010;44:405-13.
15. Demirci P, Akın Y, Midilli K, Karaaslan A, Camcıoğlu Y. Human bocavirus infection in Istanbul. Cukurova Med J. 2016;41:762-6.
16. Cebey-López M, Herberg J, Pardo-Seco J, Gómez-Carballa A, Martinón-Torres N, Salas A, et al. Viral Co-Infections in Pediatric Patients Hospitalized with Lower Tract Acute Respiratory Infections. Plos One. 2015;10:e0136526.
17. Hayden FG. Rhinovirus and the lower respiratory tract. Rev Med Virol. 2004;14:17-31.
18. Jain S, Williams DJ, Arnold SR, Ampofo K, Bramley AM, Reed C, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med. 2015;372:835-45.
19. Ghietto LM, Cámara A, Zhou Y, Pedranti M, Ferreyra S, Frey T, et al. High prevalence of human bocavirus 1 in infants with lower acute respiratory tract disease in Argentina, 2007-2009. Brazilian J Infectious Dis. 2012;16:38-44.
20. Miron D, Srugo I, Kra-Oz Z, Keness Y, Wolf D, Amirav I, et al. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? Pediatr Infect Dis J. 2010;29:7-10.
21. Chorazy ML, Lebeck MG, McCarthy TA, Richter SS, Torner JC, Gray GC. Polymicrobial acute respiratory infections in a hospital-based pediatric population. Pediatr Infect Dis J. 2013;32:460-6.
22. Cilla G, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol. 2008;80:1843-9.
23. Midulla F, Scagnolari C, Bonci E, Pierangeli A, Antonelli G, De Angelis D, et al. Respiratory syncytial virüs, human bocavirus and rhinovirus bronchiolitis in infants. Arch Dis Child. 2010;95:35-41.
24. Shi T, McLean K, Campbell H, Nair H. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta-analysis. J Glob Health. 2015;5:010408.