Is multiple viral infection a predictor of severity in children with acute bronchiolitis?

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Abstract

Background: Acute bronchiolitis is a common cause of pediatric emergency department admissions in children younger than 2.

Objectives: The study aimed to compare the outcomes and the severity of bronchiolitis in young children with multiple simultaneous respiratory virus infections to those with single virus infection and no virus identified group.

Methods: Patients with moderate and severe bronchiolitis who visited our emergency department between November 2016 and May 2017 had nasopharyngeal swab samples results tested by multiplex polymerase chain reaction were included in the study. Patients' characteristics, clinical severity of illness, and outcome (pediatric emergency department discharge, admission to ward or pediatric intensive care unit) were compared with the detected viral agents.

Results: A total of 241 patients were included in the study. The mean age was 7.8 ± 2.6 months and 147 (61%) were male. Respiratory syncytial virus was the most common detected viral agent in 108 (39%) cases followed by human rhinoviruses in 67 (24%). Respiratory syncytial virus was found more frequently in February and March ($p = 0.002$). Leukocytosis and pneumonia were more likely observed in patients with only human rhinoviruses (+) subjects ($p = 0.010$ and $p = 0.015$, respectively). Intensive care hospitalization rate (16%) was higher in patients with multiple viral agents ($p = 0.004$).

Conclusions: Respiratory syncytial virus remains the most common detected viral agent in acute bronchiolitis patients. While the pathogens detected were seasonally different, there was a significant relationship between leukocytosis, bacterial pneumonia, and detected viral agents. The disease was more severe in patients with multiple viral agents.

Keywords

Acute bronchiolitis, clinical course, viral agent

Introduction

Acute bronchiolitis (AB) is a lower respiratory tract infection (RTI) most commonly seen between 0 and 2 years and that generally manifests with wheezing, tachypnea, and intercostal, subcostal, or suprasternal retractions. The disease is diagnosed by physical examination and commonly a mild self-limited respiratory illness in infants. Although respiratory failure is a uncommon condition in the course of AB, it sometimes requires intubation and mechanical ventilation.[1]

Underlying diseases or clinical conditions such as immune deficiency, cardiac disease, chronic pulmonary disease, and prematurity are implicated in the severe nature of the condition, with the causative pathogen also being involved.[2]

Viral pathogens are generally responsible for AB. The most commonly identified agents are viruses such as

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"respiratory syncytial virus" (RSV), "human rhinovirus" (HRV), "parainfluenza virus" (PIV), "influenza virus" (INF), "human metapneumovirus" (hMPV), "adenovirus" (AdV), "human bocavirus" (HBoV), "human coronavirus" (HCoV), and "enteroviruses" (EV).[3] Since patients infected with different respiratory viruses exhibit the same clinical findings, the etiological agent can only be specifically identified through laboratory tests. Viral pathogens can be identified quickly from nasopharyngeal swab (NPS) specimens using the polymerase chain reaction (PCR) method.[4] The entry into use of multiplex PCR (m-PCR) methods capable of identifying large numbers of respiratory viruses in a single specimen has been of particular benefit in determining viral co-infections.[5,6]

The type of pathogen determined is reported to create variation in predicting a severe course of disease. The presence of more than one virus has also been reported to cause significant variation.[7] Investigation of the viral pathogen in AB is extremely important in preventing unnecessary antibiotic use and in terms of antiviral therapy or inoculation for selected patients. In addition, since AB is the most common cause of hospitalization in young infants (<12 months), a good understanding of its viral etiology and immunology can contribute to the follow-up and treatment of the disease and thus reduce total hospitalization rates in children.[8]

The purpose of our study was to determine the viral agent in patients who had AB and observed at the short stay unit in the pediatric emergency department (PED), and to investigate the effect of the viral agent(s) on the severity of the disease.

Methods

Study period and population

This study was conducted at the Emergency Department of Ege University Children's Hospital, Izmir, Turkey. In Izmir, which is the third biggest city in Turkey on the Aegean Sea, the climate is Mediterranean, with relatively mild winters, and hot and sunny summers. A total of 41,000 patients presented to the PED during the study period (November 2016–May 2017). Children who had nasal obstruction, fever, cough, and wheezing were examined, and the examination revealed that tachypnea, wheezing, rales, and rhonchus were assessed as AB. Only 2108 patients had bronchiolitis, and 274 were classified as moderate or severe. In our ED, we use the clinical score which was created by Liu et al.;[9] in this score, four clinical parameters were selected: respiratory rate, retractions, dyspnea, and auscultation.

We excluded 33 patients due to lack of NPS specimens studied or missing data. Eventually, 241 patients were included in the study. *Ten of the 33 excluded patients discharged from the ED after management, remaining 23 admitted to the general pediatric ward.*

Data collection

Only children with a first or second wheezing episode who classified as moderate or severe bronchiolitis were enrolled. Patients with three or more AB episodes and/or with congenital heart disease, cystic fibrosis, neuromuscular disease, bronchopulmonary dysplasia, or immune deficiency were excluded. Cases' demographic, clinical, and laboratory characteristics; age, sex, readmission to the ED within 24 h, prematurity, underlying disease, additional infection (pneumonia, otitis, and upper airway infection), antibiotic use, administration of invasive or non-invasive ventilation, body temperature, complete blood count (CBC), C-reactive protein (CRP), biochemical parameters, and chest x-ray; and the results of these if applicable, were investigated retrospectively from PED medical records. The follow-up of study patients after discharge (hospitalization rate, ward, pediatric intensive care unit (PICU)) and NPS results were also reviewed.

NPS specimens were sent to the laboratory inside viral transport media (UTM, Copan Diagnostics, Italy) following cold chain procedures. Specimens arriving at the laboratory and not tested immediately were stored at −80°C. Respiratory viruses and subtypes of these viruses in clinical specimens were identified using multiplex real-time PCR (Allplex Respiratory Full Panel, Seegene, South Korea). Following nucleic acid extraction (RibospinvRD viral RNA/DNA Extraction Kit, GeneAll, Seegene, South Korea) amplification was performed with dual priming oligonucleotide primers specific to respiratory viruses (INF-A, INF-B, RSV A&B, AdV, hMPV, HCoV(229E, NL63, OC43), PIV tip 1–4, HRV A/B/C, EV, HBoV1/2/3/4) using probes and single-step enzymes in line with the manufacturer's instructions. PCR products were determined using the "CFX96TM Real-time PCR System" (Bio-Rad). Internal, negative and positive controls were used in each test from the extraction stage onward. The method used was tested at least twice a year in routine application using "UK Nequas, Virus identification" external quality control software.

Cases were divided into six groups in order to establish relations between the viral agents identified and clinical manifestations. Six groups consisting of the three most commonly determined single agents, other single agents, multiple agents, and no agent determined groups were established, and the effect of these on disease severity was evaluated. Prognostic criteria were determined as discharge from the PED, and admission to the pediatric ward or PICU.

Statistical analysis

Statistical analyses were performed on SPSS v.21 software. In addition to descriptive statistical methods (mean, standard deviation, median, and interquartile range), qualitative data were compared between groups using Pearson's chi-square test (*if n value < 5, Fisher's exact test preferred*) and

one-way analysis of variance (ANOVA). Student's t test was used to compare normally distributed numerical data and the Mann–Whitney U test to compare non-normally distributed data. Odds ratio (OR) values were obtained using logistic regression analysis. Results were considered significant at $p < 0.05$ at a 95% confidence interval.

Ethical approval

The study commenced following receipt of ethical approval from the local Ethical Committee in 2017.

Results

Two hundred forty-one patients with moderate or severe bronchiolitis who had result of NPS analyzed by m-PCR were included in the study. The mean age of the patients enrolled was 7.8 ± 2.6 months, and 147 (61%) were boys. One hundred nine (45%) patients were younger than 6 months. Most patients received chest x-ray, CBC, CRP, and other biochemical parameters (87%), only chest x-ray obtained for 7 (3%), no tests performed for 23 (10%) patients. Prematurity was present in 44 (18%) of all patients, elevated body temperature in 62 (28%), leukocytosis in 59 (24%), and eosinophilia in 28 (12%). Acute otitis media (AOM) secondary to existing disease was determined in 51 cases (21%), while 14 (6%) patients were diagnosed with pneumonia and 7 (3%) with urinary tract infection (UTI). Invasive and/or non-invasive ventilation was performed to 23 (9.5%) patients. One fifth of patients were discharged from the PED, 179 (74.3%) were admitted to the pediatric ward and 13(5.4%) to the PICU. Patients' demographic, clinical, and laboratory characteristics are shown in Table 1.

At least one viral agent (with a total of 277 viruses) was determined in majority of patients (87%).

A single viral pathogen was determined in 154 (64%) of cases and multiple viral agents in 56 (23%) (two agents in 45 (19%) and three in 11 (4%)). At analysis of the single and multiple agents, RSV A-B was the most common identified agent in 108 (39%) cases, followed by HRV in 67 (24%) and hMPV in 26 (10%) (Table 2). RSV and/or HRV were determined in 159 (58%) cases, while the most common combination in multiple agent group was RSV + HRV (n = 16, 29%).

The monthly and seasonal distribution of viral agents showed that incidence of RSV infection peaked during February and March, whereas HRV peaked during November ($p = 0.002$). The rate of hospitalization to the PICU was higher in patients infected with multiple viral agents (16%) ($p = 0.004$). When compared with non-HRV viruses, patients with isolated only HRV were more likely to have leukocytosis (47%) and pneumonia (19%) ($p = 0.010$ and $p = 0.015$, respectively). (Table 3).

Associating factors of the admission to the PICU were evaluated by logistic regression analysis. Leukocytosis

Table 1. Demographic, clinical, and laboratory characteristics of the cases.

Variables	n (%) 241 (100)
Age (mean, months)	7.8 ± 2.6
<6 months of age patients	109 (45)
Sex (male)	147 (61)
Laboratory-examined patients (X-ray and/or hemogram—CRP)	218 (90)
Prematurity (+)	44 (18)
Temperature >37.5°C	62 (28)
Leucocytosis >15,000/mm³	59 (24)
Absolute neutrophil count >10,000/mm³	44 (18)
Eosinophilia >400/mm³	28 (12)
CRP >2 mg/dL	56 (23)
Secondary infection	72 (30)
Acute otitis media	1 (21)
Pneumonia	4 (6)
Urinary tract infection	7 (3)
Antibiotic administration	72 (30)
Non-invasive ventilation and/or intubation	23 (10)
Outcome	
Discharged from emergency department	49 (20)
Admission to the pediatric ward	179 (75)
Admission to the pediatric intensive care unit	13 (5)
Re-admission within 24 h	11 (22)

CRP: C-reactive protein.

Table 2. Distribution of detected viral agents.

	Single infections (%64) n (%)	Co-infections (%23) n (%)	Infections total n (%)
RSV A-B	72 (47)	36 (30)	108 (39)
HRV	37 (24)	30 (24)	67 (24)
hMPV	16 (11)	10 (8)	26 (10)
HBoV	5 (3)	20 (16)	25 (9)
INF A-B	11 (7)	11 (9)	22 (8)
PIV 1–4	5 (3)	4 (3)	9 (3)
AdV	2 (1)	6 (5)	8 (3)
HCoV	3 (2)	5 (4)	8 (3)
EV	3 (2)	1 (1)	4 (1)

RSV: respiratory syncytial virus; HRV: human rhinovirus; hMPV: human metapneumovirus; HBoV: human bocavirus; INF: influenza virus; PIV: parainfluenza virus; AdV: adenovirus; HCoV: human coronavirus; EV: enteroviruses.

and/or neutrophilia influenced hospitalization to the PICU. The most effective factor was the presence of multiple viral agent on NPS (Table 4).

All patients discharged, and no fatal complications developed in any case.

Table 3. Relationship between detected viral agents and characteristics of patients.

Characteristics	RSV mono 72 (%30)	HRV mono 37 (%15)	HMPV mono 16 (%7)	Other mono 29 (%12)	Multiple infection 56 (%23)	No agents 31 (%13)	p
Age (mean, months)	7.7±6.0	7.1±5.1	8.5±5.5	8.4±6.2	7.8±5.5	6.9±5.0	0.872
Sex (male)	46 (64)	24 (65)	7 (44)	19 (66)	29 (52)	22 (71)	0.304
Admitted months							**0.002***
November	0 (0)	6 (46)	1 (8)	0 (0)	6 (46)	0 (0)	
December	8 (28)	1 (3)	2 (7)	6 (21)	9 (31)	3 (10)	
January	14 (22)	14 (22)	7 (11)	7 (11)	16 (25)	6 (9)	
February	29 (39)	10 (13)	5 (6)	11 (14)	14 (18)	8 (10)	
March	16 (35)	2 (5)	1 (2)	3 (7)	10 (23)	12 (27)	
April	5 (35)	4 (29)	0 (0)	2 (14)	1 (7)	2 (14)	
Prematurity (+)	11 (15)	7 (19)	5 (30)	2 (7)	13 (23)	6 (19)	0.336
Temperature >37.5°C	16 (22)	9 (24)	5 (31)	10 (35)	13 (23)	9 (29)	0.809
Laboratory-examined (+)	64 (89)	35 (95)	12 (75)	27 (93)	52 (93)	28 (90)	0.309
Leucocytosis >15,000/mm³	8 (13)	16 (47)	2 (14)	7 (26)	17 (33)	9 (32)	**0.010***
ANC >10,000/mm³	6 (10)	11 (32)	2 (14)	8 (30)	12 (23)	5 (18)	0.099
Eosinophilia >400/mm³	7 (12)	8 (24)	1 (7)	4 (15)	5 (10)	3 (11)	0.463
CRP >2mg/dL, n (%)	17 (28)	13 (38)	5 (36)	7 (26)	10 (19)	4 (14)	0.245
Secondary infection							
Acute otitis media	13 (18)	7 (19)	3 (19)	9 (31)	12 (21)	7 (23)	0.464
Pneumonia	3 (4)	7 (19)	0 (0)	1 (3)	2 (4)	1 (3)	**0.015***
Urinary tract infection	2 (3)	2 (5)	1 (6)	0 (0)	2 (4)	0 (0)	0.247
Antibiotics administration (+)	18 (25)	16 (43)	4 (25)	10 (35)	16 (29)	8 (26)	0.449
Non-invasive ventilation/intubation (+)	4 (6)	3 (8)	0 (0)	1 (3)	9 (16)	6 (19)	0.061
Outcome							**0.004***
Discharged from PED	21 (29)	9 (24)	4 (25)	2 (7)	8 (14)	5 (16)	
Admission to the PW	51 (71)	27 (73)	12 (75)	26 (90)	39 (70)	24 (77)	
Admission to the PICU	0 (0)	1 (3)	0 (0)	1 (3)	9 (16)	2 (7)	
Re-admission within 24h	6 (29)	1 (11)	1 (25)	0 (0)	2 (25)	1 (20)	0.882

ANC: absolute neutrophil count; CRP: C-reactive protein; NIV: non-invasive ventilation; PED: pediatric emergency department; PW: pediatric ward; PICU: Pediatric intensive care unit, *p < 0.05.

Discussion

This study examined patients with moderate and severe AB presenting to a tertiary emergency department over one spring/winter season. Their NPS specimens studied via m-PCR method. While, RSV is still the most common detected viral agent in patients with AB, HRV is the second most widely determined agent.[10–15] Similarly in our study at least one viral pathogen was determined in 87%, the most common virus was RSV, followed by HRV.

It is known that viral RTIs have a seasonal character, particularly in regions with temperate climates, and the peak periods may change from year to year. In many studies, respiratory viruses have been reported to be active during winter (generally from November to March) in the Northern Hemisphere.[16,17] In a study from Lebanon, Finianos et al.[18] observed that RSV reached a peak in December and January, while HRV peaked in February. Similarly in our study, RSV was particularly prevalent in February and March.

Although AB does not increase the risk for serious bacterial infection, occasionally concomitant or secondary bacterial infections may occur. Of the respiratory viral infections, RSV infection seems to be the one most commonly accompanied by AOM.[19,20] In our group, the most common concomitant infection was also found as AOM.

Previous studies demonstrated that in AB, multiple viral pathogens can be detected between 9% and 44%.[21,22] In two prospective, multi-center study of patients with bronchiolitis presenting to the PED, the multiple viral pathogens detection rate reported as 22.5% and 28% and RSV+HRV combination was the most prevalent group.[23,24] Similarly in the present study, we determined multiple viral agents as 23%, and the most prevalent combination is RSV+HRV.

Since HRV is one of the most frequently determined agents in patients with AB, it is also the most widely studied pathogen.[25] In this study, we found that, the detection of HRV by PCR was associated with increase in the frequency of leukocytosis and pneumonia compared with infants with no virus detected/other groups. Calvo et al.[26]

Table 4. Factors affecting intensive care unit admission in patients with acute bronchiolitis.

Variables	PICU (–) n = 228 (%94.6)	PICU (+) n = 13 (%5.4)	Univariate analyses	Multivariate analyses	
			p	Odds ratio (95% CI)	p
Sex (M/F)	140/88	7/6	0.587	–	–
Age (mean, months)			0.440		
<3	71 (31)	4 (31)		–	–
3–12	108 (47)	8 (62)		–	–
>12	49 (22)	1 (7)		–	–
Prematurity (+)	41 (18)	3 (23)	0.644	–	–
Temperature >37.5°C	61 (27)	1 (8)	0.126	–	–
Antibiotics administration (+)	67 (30)	5 (38)	0.487	–	–
Leucocytosis >15,000/mm³	51 (25)	8 (62)	**0.004***	0.62 (0.02–14.53)	0.776
Absolute neutrophil count >10,000/mm³	36 (18)	8 (62)	**0.001***	0.01 (0.00–0.50)	**0.019***
Eosinophilia >400/mm³	27 (13)	1 (8)	0.559	–	–
CRP >2 mg/dL	50 (25)	6 (46)	0.086	–	–
Secondary infection				–	
Acute otitis media	49 (21)	2 (15)	0.600	–	–
Pneumonia	13 (6)	1 (7)	0.765	–	–
Urinary tract infection	7 (3)	0 (0)	0.521	–	–
Detected viral agents			**<0.001***		
Single infections	152 (67)	2 (15)		5.75 (0.43–77.10)	0.186
Multiple infections	47 (21)	9 (69)		323.89 (7.25–1445E4)	**0.003***
No agents	29 (13)	2 (15)		(Referans)	

PICU: Pediatric intensive care unit; CRP: C-reactive protein; ANC: absolute neutrophil count, *p < 0.05.

investigated more than 2500 cases, and determined higher leukocytosis rates and CRP elevation in patients with HRV, and reported that these patients were more frequently diagnosed with bacterial pneumonia and received antibiotics more frequently.

The detection of multiple co-incident viruses in clinical settings is becoming more common since the introduction of molecular-based multiplex PCR tests, but the clinical significance of these remains unclear. The impact of these on the disease severity is controversial. While some publications have reported that the presence of multiple viral agents exacerbates the clinical course in AB patients, others have reported that they had no impact on the disease severity.[27,28] Brand et al.[29] reported that the presence of multiple viral agents does not have an effect on clinical progression in AB. In contrast, another study from Austria conducted with a larger number of patients reported that the presence of multiple viral agents resulted in a more severe clinical course.[30] Co-infected children with multiple viral agents in our study were almost more likely to be admitted to the PICU than those with single viral infections. Compared to our study, Brand et al. developed a study with a small sample size in a small region.

The main limitations of our study are that it reflects the experience of a single center, is retrospective, and involves a single season.

In conclusion, the most commonly identified agent was RSV, followed by HRV and HMPV. The severity and course of an AB episode requiring PICU in children was correlated with the presence of more than one virus on NPS. The most common bacterial infection in AB patients was AOM. The detection of HRV changes the likelihood of leukocytosis and pneumonia in infants with bronchiolitis. Future studies are needed to investigate whether particular viruses, or combinations of viruses, influence the risk of bacterial co-infection, hospitalization, and disease severity.

Availability of data and materials

The authors may share any data included in the study.

Informed consent

Since the study was a retrospective design, informed consent was not obtained.

Ethical approval

This study was approved by the local ethics committee. To maintain patient confidentiality, the forms did not include any data that would have enabled identification of any patients. The procedures performed in this study followed the ethical standards in the Helsinki Declaration of 1964, as revised in 2008, as well as the national law.

References

1. Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. *Pediatrics* 2014; 134(5): 1474–1502.
2. Çiftel M, Biçer S and Şiraneci R. Çocuklarda Akut Bronşiolit. *JOPP Derg* 2009; 1(3): 115–123.
3. Debiaggi M, Canducci F, Ceresola ER, et al. The role of infections and coinfections with newly identified and emerging respiratory viruses in children. *Virol J* 2012; 247(9): 247.
4. Renois F, Talmud D, Huguenin A, et al. Rapid detection of respiratory tract viral infections and coinfections in patients with influenza-like illnesses by use of reverse transcription-PCR DNA microarray systems. *J Clin Microbiol* 2010; 48(11): 3836–3842.
5. Calvo C, Garcia-Garcia ML, Pozo F, et al. Clinical characteristics of human bocavirus infections compared with other respiratory viruses in Spanish children. *Pediatr Infect Dis J* 2008; 27(8): 677–680.
6. Çiçek C, Arslan A, Karakuş HS, et al. Akut solunum yolu enfeksiyonu olan hastalarda solunum virüslarının prevalansı ve mevsimsel dağılımı, 2002–2014. *Mikrobiyol Bul* 2015; 49(2): 188–200.
7. Huguenin A, Moutte L, Renois F, et al. Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. *J Med Virol* 2012; 84(6): 979–985.
8. Florin TA, Plint AC and Zorc JJ. Viral bronchiolitis. *Lancet* 2017; 389(10065): 211–224.
9. Liu L, Gallaher MM, Davis RL, et al. Use of a respiratory clinical score among different providers. *Pediatr Pulmonol* 2004; 37(3): 243–248.
10. Kanık A, Eliaçık K, Koyun B, et al. Hastanede yatan akut bronşiolit tanılı süt çocuklarında viral etiyoloji ve klinik seyre etkisi. *J Pediatr Inf* 2016; 10(4): 8.
11. Uyar M, Kuyucu N, Tezcan S, et al. Bronşiyolit tanısı alan 0-2 yaş grubu çocuklarda insan Bokavirus ve diğer solunum virüslarının sıklığının araştırılması. *Mikrobiyol Bul* 2014; 48(2): 242–258.
12. Antunes H, Rodrigues H, Silva N, et al. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. *J Clin Virol* 2010; 48(2): 134–136.
13. Chen ZR, Ji W, Wang Y, et al. Etiology of acute bronchiolitis and the relationship with meteorological conditions in hospitalized infants in China. *J Formos Med Assoc* 2014; 113(7): 463–469.
14. Azkur D, Ozaydın E, Dibek-Mısırlıoğlu E, et al. Viral etiology in infants hospitalized for acute bronchiolitis. *Turk J Pediatr* 2014; 56(6): 592–596.
15. Miller EK, Gebretsadik T, Carroll KN, et al. Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. *Pediatr Infect Dis J* 2013; 32(9): 950–955.
16. Morikawa S, Kohdera U, Hosaka T, et al. Seasonal variations of respiratory viruses and etiology of humanrhinovirus infection in children. *J Clin Virol* 2015; 73(6): 14–19.
17. Trenholme AA, Best EJ, Vogel AM, et al. Respiratory virus detection during hospitalisation for lower respiratory tract infection in children under 2 years in South Auckland, New Zealand. *J Paediatr Child Health* 2017; 53(6): 551–555.
18. Finianos M, Issa R, Curran MD, et al. Etiology, seasonality, and clinical characterization of viral respiratory infections among hospitalized children in Beirut, Lebanon. *J Med Virol* 2016; 88(11): 1874–1878.
19. Kanra G, Tezcan S and Yilmaz G. Respiratory syncytial virus epidemiology in Turkey. *Turk J Pediatr* 2005; 47(4): 303–308.
20. Karppinen S, Toivonen L, Schuez-Havupalo L, et al. Interference between respiratory syncytial virus and rhinovirus in respiratory tract infections in children. *Clin Microbiol Infect* 2016; 22(2): e1–e6.
21. Mansbach JM, McAdam AJ, Clark S, et al. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. *Acad Emerg Med* 2008; 15(2): 111–118.
22. Nascimento MS, Souza AV, Ferreira AV, et al. High rate of viral identification and coinfections in infants with acute bronchiolitis. *Clinics* 2010; 65(11): 1133–1137.
23. Wishaupt JO, Van der ploeg T, Groot R, et al. Single- and multiple viral respiratory infections in children: disease and management cannot be related to a specific pathogen. *BMC Infect Dis* 2017; 17(1): 62.
24. Chen YW, Huang YC, Ho TH, et al. Viral etiology of bronchiolitis among pediatric inpatients in northern Taiwan with emphasis on newly identified respiratory viruses. *J Microbiol Immunol Infect* 2014; 47(2): 116–121.
25. Jacobs SE, Lamson DM, George KS, et al. Human rhinoviruses. *Clin Microbiol Rev* 2013; 26(1): 135–162.
26. Calvo C, Garcia-Garcia ML, Pozo F, et al. Respiratory syncytial virus coinfections with rhinovirus and human bocavirus in hospitalized children. *Medicine* 2015; 94(42): e1788.
27. Luchsinger V, Ampuero S, Palomino MA, et al. Comparison of virological profiles of respiratory syncytial virus and rhinovirus in acute lower tract respiratory infections in very young Chilean infants, according to their clinical outcome. *J Clin Virol* 2014; 61(1): 138–144.
28. Richard N, Komurian-Pradel F, Javouhey E, et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. *Pediatr Infect Dis J* 2008; 27(3): 213–217.
29. Brand HK, de Groot R, Galama JM, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. *Pediatr Pulmonol* 2012; 47(4): 393–400.
30. Aberle JH, Aberle SW, Pracher E, et al. Single versus dual respiratory virus infections in hospitalized infants. Impact on clinical course of disease and interferon-gamma response. *Pediatr Infect Dis J* 2005; 24(7): 605–610.