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ORIGINAL ARTICLE

Investigation of factors by real-time polymerase chain reaction analysis in hospitalized patients with acute lower respiratory tract infections

[1]Ceren YAPAR GÜMÜŞ
[2]Feyza Mediha YILDIZ

[1]Department of Pediatrics, Ordu University Faculty of Medicine, Ordu, Turkey

[2]Department of Pediatrics, University of Health Sciences, Turkey. Istanbul Zeynep Kamil Maternity and Children's Diseases Health Training and Research Center, Istanbul, Turkey

ORCID ID
CYG : 0000-0001-6349-2514
FMY : 0000-0002-8684-0101

ABSTRACT

Objective: Acute bronchiolitis and/or pneumonia are generally referred to as lower respiratory tract infections (LRTI). It was aimed to investigate the agents in LRTI, which is one of the most important causes of childhood deaths, by real-time polymerase chain reaction (PCR) method from the nasopharyngeal aspirate.

Material and Methods: In our study, chest radiographs, clinical, demographic, laboratory characteristics, and disease agents obtained by real-time PCR were examined in patients aged 1 month to 18 years who were hospitalized with a prediagnosis of LRTI in the pediatric service during the year 2019.

Results: The patients' mean age was 25.89±36.72 months, and 57.05% (n=279) of the study group were male. Patients are grouped monthly; of the study group, 69.3% were between the ages of one and 24 months, and 16.0% were between the ages of 24 and 60 months. In the study group, 38% (n=186) of 489 patients had a fever. In 93.9% of cases, a cough was present. Of the 489 patients in the study group, 175 (35.7%) had no detectable causative agent, while 314 (64.3%) had one or more. After analyzing individual factors, 28.34% of the study group had Rhinovirus as the causative agent.

Conclusion: The most frequent cause of LRTI was determined to be Rhinovirus in our investigation, in contrast to the general literature in our nation. Other data appear to be generally compatible with national and international literature.

Keywords: Lower respiratory tract infections, pediatric, RT-PCR.

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Correspondence: Ceren YAPAR GÜMÜŞ, MD. Ordu Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Ordu, Türkiye.
Tel: +90 452 226 52 14 **e-mail:** cerenyapar91@gmail.com

INTRODUCTION

Pneumonia, bronchopneumonia, and acute bronchiolitis are commonly referred to as lower respiratory tract infections (LRTIs). Acute bronchiolitis is the most common presentation of LRTI in the first years of life. In this age period, almost one out of every three children is diagnosed with acute bronchiolitis based on clinical findings, and hospitalization is considered appropriate in approximately 2–3% of children.[1] In a clinical study conducted in Türkiye, it was reported that the rate of hospitalization due to acute LRTI in children under two years of age was 20.5 per 1000 children and that half of these hospitalizations were acute bronchiolitis cases and the causative agent detected in 41% of these cases was Respiratory syncytial virus (RSV).[2] LRTI was responsible for 13.9% of the 5.30 million deaths in children under five in 2019, making it the most common cause of death for children aged one to fifty-nine months.[3] In children under two years of age, viral agents are responsible for 80% of the disease.[4] Among viral pathogens, RSV ranked first with 40%, followed by Adenovirus, Bocavirus, Parainfluenza, Rhinovirus, Human metapneumovirus (HMPV), Coronavirus, Parainfluenza, Influenza A and B.[5] Studies on the etiology and clinical outcomes of LRTI, which have significant morbidity and mortality rates, are of utmost importance. For this reason, in our study, we aimed to define the demographic and clinical characteristics of pediatric patients aged between 1 month and 18 years who were hospitalized with a prediagnosis of LRTI in the pediatric service of our hospital and to investigate the causative agents of the disease.

MATERIAL AND METHODS

Study Design: The study population consisted of patients between the ages of one month to 18 years who applied to a tertiary care training and research hospital in Istanbul in 2019 and were hospitalized in the pediatric service with a prediagnosis of LRTI and met the inclusion criteria. The medical records of the patients were retrospectively reviewed and chest radiographs taken during hospitalization were evaluated by an experienced pediatric radiologist. The number of study participants when the inclusion/exclusion criteria were applied was 489.

The inclusion criteria were as follows: The patient was between the ages of 1 month and 18 years, did not have any additional cardiac disease, and was hospitalized and treated with a prediagnosis of LRTI in the pediatric service of our hospital.

Exclusion criteria: Age <1 month, presence of additional cardiac disease, voluntary refusal of hospitalization by the patient's family/legal guardian.

The agents detected by real-time PCR in our hospital are Klebsiella pneumoniae, Influenza A, Influenza B, Parainfluenza Type 1, Parainfluenza Type 2, Parainfluenza Type 3, Parainfluenza Type 4, Mycoplasma pneumoniae, Enterovirus, HPMV, RSV A/B, Bocavirus, Rhinovirus, Coronavirus, Pandemic H1N1, Seasonal H1N1. The results of real-time PCR tests taken at the time of hospitalization of patients hospitalized with a prediagnosis of LRTI were reported as positive and negative.

Age groups were categorized as >60 months, 24–60 months, and 1–24 months. Initially, patients were classified as either causative or non-causative based on real-time PCR detection of the causative agent. The group in which the causative agent was detected was divided into subgroups as two and three causative agents detected/single causative agent detected.

Chest X-ray findings of the study population were evaluated by a pediatric radiologist. Findings were recorded as normal, atelectasis, peribronchial infiltration, increased aeration, reticulonodular infiltration, consolidation, increased vascular central markings, and blunting of the sinus. The study group was further subdivided into two subgroups: those with both of these findings and those with more than two radiologic findings.

According to medical history, clinical course, physical examination, and imaging findings, the study group was divided into four diagnostic groups: pneumonia, lobar pneumonia, bronchopneumonia, and bronchiolitis.

Statistical Analysis

The Statistical Package for the Social Sciences, version 22.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the study data. Descriptive data were presented as frequency and numerical data were presented as median (min–max) or mean±SD. The Kolmogrov-Smirnov test was used to evaluate the conformity of the data to normal distribution.

The chi-square test was used for the comparison of categorical data between groups. Pearson test or Fisher exact test was preferred according to the number of groups. The Mann-Whitney U test was used for intergroup comparison of numerical data not conforming to a normal distribution, and the independent sample t-test was used for intergroup comparison of numerical data conforming to a normal distribution. A logistic regression test was used to determine the predictive function and cause-effect relationship of the presence of a viral agent using binary or multiple variables.

Data are presented at a 95% confidence interval, $p<0.05$ was considered statistically significant, and $p=0.000$ values were presented as $p<0.001$.

Ethics Statement

The study was approved by the Zeynep Kamil Women and Children's Diseases Training and Research Hospital Clinical Research Ethics Committee on 21.08.2019 with decision number 82. This study was conducted by adhering to the Declaration of Helsinki.

RESULTS

Main Characteristics of the Study Group: The median age of the patients was 8.00 (1–196) months and the mean age was 25.89±36.72 months. There were 42.95% (n=210) females and 57.05% (n=279) males in the study group. When the patients were grouped based on months, 16.0% of the study group was between 24–60 months and 69.3% was between 1–24 months. When the patients were evaluated according to the months of admission to the hospital, it was found that admissions in January and December were more frequent than the other months. While 12.7% (n=62) of the patients were admitted in January, 19% (n=93) were admitted in December. Table 1 shows the study group's laboratory parameters.

Table 1: Laboratory parameters of the study group

Parameters	Mean	Standard deviation	Median	Minimum	Maximum
Hb (g/dL)	11.20	1.46	11.1	6.90	17.7
WBC (10^3/µL)	12.44	5.68	11.24	1.06	36.28
PLT (10^3/µL)	366.34	140.62	346.00	21.00	1011.00
CRP (mg/dL)	2.35	4.15	0.69	0.2	34.00

Hb: Hemoglobin; WBC: White blood cell count; PLT: Platelet count; CRP: C-reactive protein.

Fever, cough, sibilant rhonchi, prolonged expiratory phase of respiration, tachypnea, and crepitant rales were all noted as the patient's clinical findings upon admission. Cough was present in 93.9% of the 489 patients and constituted the most common finding.

The median duration of hospitalization in the study group was 5.00 (1–82) days and the mean was 7.89±5.48 days.

In the study group, one or more agents were detected in 314 (64.3%) of 489 patients, while no agent was detected in 175 (35.7%). When single agents were analyzed, seasonal H1N1 Influenza A was detected in 1 of 314 patients, Parainfluenza 2 in 1, Parainfluenza 4 in 2, Pandemic H1N1 Influenza A in 3, Enterovirus in 3, Influenza A in 3 patients, Coronavirus in 4 patients, Klebsiella pneumoniae in 5 patients, Mycoplasma pneumoniae in 7 patients, Parainfluenza 1 in 7 patients, Influenza B in 7 patients, Parainfluenza 3 in 9 patients, Bocavirus in 16 patients (5.09%), HMPV in 17 patients (5.41%), RSV in 69 patients (21.97%), and Rhinovirus in 89 patients (28.34%). While there were 16 patients with 3 or more agents, there were 55 patients (17.5%) with two viral agents. When the radiologic findings during hospitalization were analyzed, no pathologic radiologic findings were found in 36.2% of the cases (n=177). The most common single pathologic radiologic finding was consolidation with 20.2% (n=99), followed by peribronchial infiltration (n=68). The frequencies of radiologic findings are given in detail in Table 2.

Regarding the diagnosis, it was determined that 38.2% (n=187) of the patients had bronchiolitis and 41.7% (n=204) had bronchopneumonia. 67 patients were diagnosed with pneumonia and 31 with lobar pneumonia.

Table 2: Imaging findings of the study group

Imaging findings	Study group (n=489)	
	n	%
Normal	177	36.2
Single radiologic finding		
Atelectasis	14	2.9
Peribronchial infiltration	68	13.9
Increased aeration	8	1.6
Reticulonodular infiltration	12	2.5
Consolidation	99	20.2
Total	201	41.10
Dual Radiologic Findings		
Peribronchial + reticulonodular infiltration	5	1.0
Atelectasis + peribronchial infiltration	13	2.7
Consolidation + peribronchial İnfiltrasyon	29	5.9
Atelektasis + Retikülonodüler infiltration	5	1.0
Peribronchial infiltration + increased aeration	1	0.2
Atelectasis + consolidation	15	3.1
Consolidation + reticulonodular infiltration	6	1.2
Sinus Blunting + consolidation	5	1.0
Sinus Blunting + atelectasis	4	0.8
Sinus Blunting + peribronchial infiltration	2	0.4
Total	86	17.6
>2 radiologic findings	25	5.1

Statistical Analysis of Agents Detected by Real-time PCR

Eighteen (10.28%) of 175 patients, whose pathogen was not detected as a result of PCR, were admitted in summer, 30 (17.14%) in autumn, 37 (21.14%) in spring, and 90 (51.42%) in winter. Of the 243 patients who were found to have a single respiratory tract infection agent as a result of PCR, 22 (9.05%) were admitted to the hospital in summer, 54 (22.22%) in autumn, 77 (31.68%) in spring, and 90 (37.03%) in winter. Of 71 patients with more than one respiratory tract infection factor detected by PCR, 9 (12.67%) were admitted to the hospital in summer, 18 (25.35%) in spring, 20 (28.16%) in autumn, and 24 (33.8%) in winter. The frequency of admission to the hospital in the winter season in cases with no causative agents in PCR results is significantly higher than in patients with single or more than one respiratory tract agent (p=0.025).

Rhinovirus (68.51%) was detected in 37 of 54 patients admitted to hospital in the autumn season. No patient had RSV as the single respiratory tract infection agent this season.

Of the 90 patients admitted to the hospital in the winter season, RSV was detected in 53 (58.88%), Rhinovirus in 10 (11.11%), Bocavirus in 9 (10%), Pandemic H1N1-A, Influenza A, Mycoplasma pneumoniae, and HMPV in 3 cases each (3.33%). Parainfluenza 1 and Coronavirus were detected in 2 patients each.

Table 3: The relationship between laboratory parameters of cases with and without causative agents

Laboratory parameters	Causative agent	No causative agent	p
Hb (g/dL), Mean±SD	11.11±1.41	11.37±1.53	0.059
WBC (10^3/µL), Mean±SD	12.42±5.93	12.48±5.23	0.911
PLT (10^3/µL), Mean±SD	365.74±13.73	367.43±14.67	0.899
CRP (mg/dL), Median (Min–Max)	0.51 (0.2–25.76)	1.16 (0.20–34.00)	**0.003**

Hb: Hemoglobin; WBC: White blood cell count; PLT: Platelet count; CRP: C-reactive protein; Min: Minimum; Max: Maximum; SD: Standard deviation.

Of the 77 patients admitted in the spring season and the only respiratory tract infection agent detected by PCR, Rhinovirus was detected in 32 (41.55%), RSV in 16 (20.77%), HMPV in 8 (10.39%), and Influenza B in 7 (9.09%).

Rhinovirus was detected in 10 (45.5%), Bocavirus in 2 (9.09%), Parainfluenza Type 3, Mycoplasma pneumoniae, and Klebsiella pneumoniae in 3 (13.63%) of 22 patients admitted in the summer season. In this season, no patient had RSV as the single respiratory tract infection agent.

The age of 74.9% (n=182) of the 243 cases in which a single respiratory tract infection agent was detected by PCR was between 1 and 24 months. The age of 80% (n=4) of the patients with Klebsiella pneumoniae was between 1–24 months. 94.7% (n=18) of all Parainfluenza cases, 100% (n=4) of Coronavirus cases, 92.8% (n=64) of RSV cases, and 82.4% of HMPV cases were between 1–24 months of age.

Between the age groups of patients with one respiratory tract infection agent and patients with multiple respiratory tract infection agents, there was no statistically significant difference (p=0.701).

The frequency of negative PCRs obtained during hospitalization in patients with a diagnosis of lobar pneumonia was statistically significantly higher than in other patient groups (p<0.001).

Compared to other months, there was a significant increase in the probability of detecting a viral agent in September and January (p<0.001).

The CRP median of the group with no causative agent was statistically significantly higher than the CRP median of the group with a causative agent (p=0.003). The relationship between laboratory parameters of cases with and without causative agents is shown in Table 3.

The mean age (months) of the cases in which the agent was detected was significantly lower than the mean age of the cases in which the agent was not detected (20.54 vs. 35.51 months) (p<0.001).

The median duration of hospitalization in the study group was 5.00 (1–82) days, while the mean was 7.89±5.48 days. The mean duration of hospitalization in cases without causative agents was statistically significantly higher than in cases with causative agents (9.01 vs. 7.26; p<0.001).

Statistical Analysis of Data by Age Groups

The study group was categorized as >60 months (n=72), 24–60 months (n=78), and 1–24 months (n=339). The frequency of physical examination findings and the relationship between clinical findings and age groups are shown in Table 4.

The frequency of crepitant rales and fever was significantly lower in patients aged 1–24 months in the study group compared to other age groups (p<0.001), whereas the frequency of tachypnea, prolonged expiration, and rhonchi was significantly higher (p<0.001).

The frequency of radiologic findings according to age groups is presented in Table 5.

When the presence of pathologic imaging findings was analyzed according to age groups, it was observed that the frequency of pathologic imaging findings in children increased significantly with increasing age. While 93.1% of children older than 60 months had pathologic findings, 53.7% of children aged 1–24 months had at least one pathologic finding (p<0.001).

When the median length of hospitalization was analyzed according to age groups, the median length of hospitalization for children between 24–60 months and 1–24 months was 7 days (7 (4–24) days for 24–60 months; 7 (1–82) days for 1–24 months), while the median length of hospitalization for children over 60 months was 8 (3–53) days. Compared to other age groups, the median length of hospitalization for children over 60 months was found to be statistically significantly higher (p=0.009).

When the relationship between diagnoses and age groups was evaluated, pneumonia constituted 36.1% of the diagnoses in children older than 60 months, 15.4% in children aged 24–60 months, and 8.6% in children aged 1–24 months. There was a statistically significant correlation between the increase in the frequency of pneumonia and increasing age (p<0.001).

DISCUSSION

In the pediatric population, viral respiratory infections are a serious public health problem and an important cause of morbidity and mortality.[6,7] It has been emphasized that the prevalence of viral respiratory infections, which are responsible for 5 million child deaths annually in children under five years of age, is 2–3 times higher in children than in adults in developing countries.[8,9]

Viruses are the main pathogens in LRTI.[10] Real-time PCR analysis is a sensitive, rapid, and specific test for the detection of respiratory viruses, unlike conventional diagnostic methods to date. Literature studies show that the sensitivity of real-time PCR in detecting respiratory tract pathogens ranges between 95% and 100%, and the specificity between 91.3% and 100%.[11]

Table 4: The frequency of physical examination findings and the relationship between clinical findings and age groups

Description	1–24 months (n)	24–60 months (n)	>60 months (n)	p
Fever				**<0.001**
Yes	100	38	48	
No	239	40	24	
Cough				0.67
Yes	319	74	66	
No	20	4	6	
Sibilant rhonchi				**<0.001**
Yes	141	27	13	
No	198	51	59	
Prolonged expiratory phase of respiration				**<0.001**
Yes	288	57	33	
No	51	21	39	
Tachypnea				**<0.001**
Yes	215	29	20	
No	124	49	52	
Crepitant rales				**<0.001**
Yes	156	49	49	
No	189	29	23	

Table 5: Frequency of imaging findings by age groups

Radiological findings	1–24 months	24–60 months	>60 months	Total
Normal	157	15	5	177
Atelectasis	9	5	0	14
Peribronchial infiltration	56	9	3	68
Increased aeration	7	1	0	8
Reticulonodular infiltration	8	0	4	12
Consolidation	37	26	36	99
Dual findings	51	15	20	86
>2 findings	14	7	4	25
Total	339	78	72	**489**

In our study, we investigated the disease agents by real-time PCR, and the demographic, clinical, laboratory, and radiological imaging characteristics of patients aged between 1 month and 18 years admitted to a tertiary care training and research hospital in Istanbul in 2019 and hospitalized in the pediatric service with a prediagnosis of LRTI.

Although the pathogens of viral LRTI vary according to years and seasons, RSV is the most common agent in the literature. In a 2008 research performed by Mansbach et al.,[12] RSV was detected in 72% of the study group. In a study by Sarkar et al.,[13] in children with LRTI, RSV was found to be the causative agent in 40.68% of children. If we look at the studies in Türkiye, the prevalence of RSV ranges between 20% and 50%.[14–16] In our study, RSV was found to be a pathogen in 21.97% of 314 cases with causative agents. However, contrary to the literature, the major causative agent in our study was Rhinovirus (28.34%). According to the literature, these differences in rates may be attributed to the frequency of hospitalization, season of hospitalization, and age group.

In this study, RSV was detected as the predominant causative agent in the winter season, and Rhinovirus was found to be the predominant causative agent except in the winter season. Rhinovirus

dominance was also observed in the autumn season in the study performed by Jiang et al.[17] Although the causes of respiratory tract infections have their specific seasons, subgroup analysis did not provide a statistically significant result because there were not enough numbers for other subgroups in our study.

Analysis of the correlation between agent positivity and symptoms revealed that, aside from tachypnea frequency, there was no statistically significant difference between the agent's presence and other symptoms. Kamata et al.[18] found that patients infected with RSV were more likely to have rhonchi. In the study by Bharaj et al.,[19] no significant difference was found in the frequency of symptoms between cases with and without the causative agent. Similar results were obtained in the study by Papadopoulos et al.[20] Our study was found to be consistent with the literature in this regard.

In our study, it was shown that the median CRP of the group with no causative agents was statistically significantly higher than the group with the causative agent. This situation has revealed the prediction that some bacterial pathogens that cannot be demonstrated by PCR may also accompany the clinical picture.

The mean length of hospitalization of patients in whom the causative agent was not detected was statistically significantly higher than that of patients in whom the causative agent was detected (9.01 vs. 7.26; $p<0.001$). This was attributed to the advanced investigations performed in patients in whom the causative agent was not detected and the longer duration of treatment in LRTI cases caused by bacterial infections that could not be detected by the PCR method.

When the study population is analyzed according to age groups, it provides valuable information to make new contributions to the literature. In patients aged 1–24 months, the frequency of crepitant rales and fever was statistically significantly lower, and the frequency of prolonged expiration, rhonchi, and tachypnea was noticeably higher in the study group when compared to other age groups. In previous studies, it was observed that agent frequency analyses were performed according to age groups, and the frequency of findings was evaluated according to the agents. Therefore, this result is important in terms of its contribution to the literature.

When the presence of pathologic chest radiograph findings was analyzed according to age groups, it was observed that the frequency of pathologic findings increased significantly with increasing age in the study population. This can be evaluated together with the relationship between age and the causative agent detected. Further studies on this subject are needed.

It was found that the median duration of hospitalization was statistically significantly higher in patients aged over 60 months compared to other age groups. This result is important for pediatricians in the management of lower respiratory tract infections that require a multidisciplinary approach.

The first limitation of our study is its retrospective design. In this study, in which hospitalizations in the period before the COVID-19 pandemic period were evaluated, Coronavirus cases were few. Due to the statistically insufficient number of all agents found in the respiratory viral panel, we could not effectively compare our study with literature studies examining clinical features associated with specific viral respiratory tract pathogens, which constitutes a shortcoming of this study.

CONCLUSION

Contrary to the general literature in our country, Rhinovirus was found to be the most common cause of LRTI in our study. Other data are largely consistent with the national and international literature. Our study may guide planning general patient management according to age groups. The findings of this investigation will offer more information about the epidemiology and appropriate management of LRTIs in our country and help to reduce morbidity and mortality rates related to LRTIs.

Statement

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Ethics Committee Approval: The University of Health Sciences, Turkey. Istanbul Zeynep Kamil Maternity and Children's Diseases Health Training and Research Center Clinical Research Ethics Committee granted approval for this study (date: 21.08.2019, number: 82).

Author Contributions: Concept – CYG, FMY; Design – CYG, FMY; Supervision – CYG; Resource – CYG; Materials – CYG; Data Collection and/or Processing – CYG; Analysis and/or Interpretation – CYG; Literature Search – CYG, FMY; Writing – CYG, FMY; Critical Reviews – CYG, FMY.

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REFERENCES

1. National Institute for Health and Care Excellence. Bronchiolitis in children: Diagnosis and management guidance. 2021. Available at: https://www.nice.org.uk/guidance/ng9/evidence. Accessed Sep 30, 2022.
2. Hacımustafaoğlu M, Celebi S, Bozdemir SE, Ozgür T, Ozcan I, Güray A, et al. RSV frequency in children below 2 years hospitalized for lower respiratory tract infections. Turk J Pediatr 2013;55:130–9.
3. Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, et al. Global, regional, and national causes of under-5 mortality in 2000-19: An updated systematic analysis with implications for the Sustainable Development Goals. Lancet Child Adolesc Health 2022;6:106–15.
4. Hamano-Hasegawa K, Morozumi M, Nakayama E, Chiba N, Murayama SY, Takayanagi R, et al. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. J Infect Chemother 2008;14:424–32.
5. Drummond P, Clark J, Wheeler J, Galloway A, Freeman R, Cant A. Community acquired pneumonia--a prospective UK study. Arch Dis Child 2000;83:408–12.
6. Heikkinen T. Respiratory viruses and children. J Infect 2016;72(Suppl):S29–33.
7. Liolios L, Jenney A, Spelman D, Kotsimbos T, Catton M, Wesselingh S. Comparison of a multiplex reverse transcription-PCR-enzyme hybridization assay with conventional viral culture and immunofluorescence techniques for the detection of seven viral respiratory pathogens. J Clin Microbiol 2001;39:2779–83.
8. Ozdemir S, Ozturk TC, Metiner Y, Ak R, Ocal O. Evaluation of the prescriptions written for upper respiratory tract infections. North Clin Istanb 2015;2:107–14.

9. Templeton KE. Why diagnose respiratory viral infection? J Clin Virol 2007;40(Suppl 1):S2–4.
10. Tregoning JS, Schwarze J. Respiratory viral infections in infants: Causes, clinical symptoms, virology, and immunology. Clin Microbiol Rev 2010;23:74–98.
11. Mahony JB. Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 2008;21:716–47.
12. Mansbach JM, Piedra PA, Teach SJ, Sullivan AF, Forgey T, Clark S, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Arch Pediatr Adolesc Med 2012;166:700–6.
13. Sarkar S, Ratho RK, Singh M, Singh MP, Singh A, Sharma M. Comparative analysis of epidemiology, clinical features, and cytokine response of respiratory syncytial and human metapneumovirus ınfected children with acute lower respiratory ınfections. Jpn J Infect Dis 2022;75:56–62.
14. Sancakli O, Yenïgün A, Kırdar S. Results of polymerase chain reaction in nasopharyngeal swab specimens of patients with lower respiratory tract infection. J Pediatr Inf 2012;6:84–9.
15. 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 20137;39:22.
16. Akçalı S, Yılmaz N, Güler Ö, Şanlidağ T, Anıl M. The frequency of respiratory tract viral agents in children with lower respiratory tract infections. Turk Arch Pediatr 2013;48:215–20.
17. Jiang H, Yang T, Yang C, Lu Y, Yi Z, Zhang Q, et al. Molecular epidemiology and clinical characterization of human rhinoviruses circulating in Shanghai, 2012-2020. Arch Virol 2022;167:1111–23.
18. Kamata K, Thein KN, Di Ja L, Win NC, Win SMK, Suzuki Y, et al. Clinical manifestations and outcome of viral acute lower respiratory infection in hospitalised children in Myanmar. BMC Infect Dis 2022;22:350.
19. Bharaj P, Sullender WM, Kabra SK, Mani K, Cherian J, Tyagi V, et al. Respiratory viral infections detected by multiplex PCR among pediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. Virol J 2009;6:89.
20. Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 2002;165:1285–9.