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Original Article

Retrospective evaluation of viral respiratory tract infections in a university hospital in Ankara, Turkey (2016-2019)

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Abstract

Introduction: Viruses are responsible for two-thirds of all acute respiratory tract infections. This study aims to retrospectively detect respiratory tract viruses in patients from all age groups who visited the hospital.

Methodology: A total of 1592 samples from 1416 patients with respiratory tract symptoms were sent from several clinics to the Molecular Microbiology Laboratory at Gazi University Hospital from February 2016 to January 2019. Nucleic acid extraction from nasopharyngeal swabs, throat swabs or bronchoalveolar lavage (BAL) samples sent to our laboratory was done using a commercial automated system. Extracted nucleic acids were amplified by a commercial multiplex-real time Polymerase Chain Reaction (PCR) method, which can detect 18 viral respiratory pathogens.

Results: Among 1592 samples, 914 (57.4%) were positive for respiratory viruses. The most prevalent were rhinovirus (25.2%) and influenza A virus (12.1%), the least prevalent was the bocavirus (2.6%). Rhinovirus was the most detected as a single agent (21.2%, 194/914) among all positive cases, followed by coronavirus (9.3%, 85/914). The detection rates of coronavirus, human adenovirus, respiratory syncytial virus A/B, human parainfluenza viruses, human metapneumovirus-A/B, human parechovirus, enterovirus and influenza B virus were 9.9%, 8%, 7.7%, 5%, 3.4%, 3.1%, 3%, and 2.8%, respectively.

Conclusions: The most detected viral agents in our study were influenza A virus and rhinovirus. Laboratory diagnosis of respiratory viruses is helpful to prevent unnecessary antibiotic use and is essential in routine diagnostics for antiviral treatment. Multiplex Real-time PCR method is fast and useful for the diagnosis of viral respiratory infections.

Key words: Respiratory viruses; rhinovirus; influenza virus; coronavirus; multiplex real-time PCR.

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Introduction

Acute respiratory tract infections (ARTIs) are a conspicuous public health problem globally, causing significant morbidity and mortality among people of all age groups [1]. It is estimated that acute respiratory tract infections cause 3.9 million deaths per year and are among the top five causes of mortality globally. These infections especially affect children, older people, and patients with chronic illnesses. Respiratory viruses cause nearly half of the community-acquired pneumonia (CAP) cases in children, more than 90% of the bronchiolitis cases in infants, and 85-95% of the asthma exacerbations in children. In adults, they are responsible for 30-50% of CAP cases, 80% of asthma exacerbations, and 20-60% of chronic obstructive pulmonary disease (COPD) exacerbations [2]. Therefore, respiratory tract viruses cause a significant burden to the healthcare systems and economic costs to the society as a result of medical expenses and workforce losses [2].

Many pathogens such as bacteria, viruses, mycoplasma, chlamydia, and fungi can cause ARTIs, however, viruses are responsible for the majority of ARTIs [3]. Lower respiratory tract infections (LRTIs) are defined as one of the major causes of morbidity and mortality in critically ill patients. The most commonly detected viruses can vary based on patient population, season, and location. The most common virus types in

patients with LRTIs include influenza virus, rhinovirus (RV), coronavirus (CoV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza virus (hPIV), and adenovirus (hAdV) [3,4].

Although viruses or atypical pathogens are the common pathogens causing unexplained pneumonia, their clinical manifestations can complicate the diagnosis of the causative pathogens, which may cause unnecessary usage of antimicrobials. Therefore, rapid and precise diagnosis of the causative agents is critical for the prompt management of unexplained pneumonia [5]. Various methods such as viral culture, hemagglutination inhibition assay, enzyme immunoassay, rapid antigen tests and molecular methods that can detect viral nucleic acids for the diagnosis of respiratory tract viruses are available. Although culture methods are still the gold standard, molecular methods such as multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) are easier, quicker, and more sensitive and specific than viral culture methods. Additionally, they enable detection of a broader panel of viruses and coinfections [3,4,6].

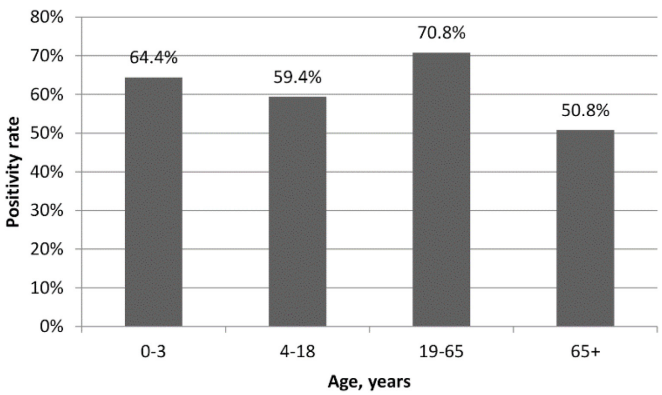
This study evaluated the distribution and characteristics of viral respiratory infection agents using multiplex RT-PCR method in patients admitted with respiratory tract infection symptoms at a university hospital during a period of three years.

Methodology

Study Design

A total of 1416 patients (1186 outpatients, 230 inpatients), aged 0-101 years, who came to Gazi University Faculty of Medicine Hospital (a tertiary care hospital with 1007 hospital beds) with suspicion of viral respiratory tract infection, from February 2016 to January 2019 were included in the study. Samples of nasopharyngeal, throat, or nasal swabs that were collected from the patients were sent to the laboratory in viral transport medium (UTM-RT transport, Copan Diagnostics, Copan, Italy); bronchoalveolar lavage (BAL) samples were sent to the laboratory in a sterile transport container.

Figure 1. Positivity rates of patients according to age groups.



Viral Identification

Viral nucleic acid extraction was done by using a commercial automated system (EZ1 Virus Mini Kit, Qiagen, Stockach, Germany). Extracted nucleic acids were qualitatively amplified by a multiplex RT-PCR method (FTD Respiratory Pathogens 21, Fast Tract Diagnostics, Esch-sur-Alzette, Luxembourg) that can detect 18 viral respiratory pathogens (RV, Influenza A viruses-IAV, Influenza A/H1N1, Influenza B virus, CoV [229E, HKU1, NL63, OC43], AdV, RSV A/B, hPIV [1,2,3,4], hMPV-A/B, Parechovirus- HPeV, Enterovirus-EV, Bocavirus-BoV). The samples were run in five separate tubes according to the manufacturer's instructions. The results of influenza A, B, H1N1, and RV were analyzed in the group 'flu', CoV NL63, 229E, OC43, and HKU1 in the group 'cor', hPIV 2, 3, 4 and internal control in the group 'para', hPIV 1, hMPV-A/B, hBoV in the group 'bo', and RSV A/B, hAdV, EV, hPeV in the group 'rs'.

Statistical Analysis

The data were analyzed by using IBM SPSS Statistics version 20.0 (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). The associations between age groups were analyzed by Pearson χ^2 Test.

Results

A total of 1592 respiratory tract samples of 1416 patients, 637 of whom were female and 779 males, were sent to the Molecular Virology Laboratory from various clinics. More than one sample was collected from some patients. Seven hundred and three (44%) of the samples were from various pediatrics departments (transplantation, hematology, oncology, emergency, etc.), 173 (11%) from adult hematology, 167 (10.5%) from infectious diseases, 131 (8%) from chest diseases, 70 from nephrology (4%), 64 (4%) from adult transplantation units, and the rest (18.5%) from various clinics (internal diseases, general surgery, neurology, gynecology and obstetrics, oncology, urology, etc.). Some of the patients had developed respiratory infections during hospital stay. Others had underlying diseases, such as cancer, and hematologic diseases, and

Table 1. Distribution of viral agents according to age group.

Viruses n (%)	0-3 yrs	4-18 yrs	19-65 yrs	65+ yrs	Total	*p*[a]
Respiratory synctial virus	58 (44.6%)	22 (16.9%)	30 (23.1%)	20 (15.4%)	130 (100%)	< 0.001
Influenza viruses	29 (12.8%)	63 (27.9%)	78 (34.5%)	56 (24.8%)	226 (100%)	< 0.001
Parainfluenza viruses	20 (28.2%)	22 (31%)	18 (25.4%)	11 (15.5%)	71 (100%)	0.265
Rhinovirus	87 (24.1%)	109 (30.2%)	108 (29.9%)	57 (15.8%)	361 (100%)	< 0.001
Adenovirus	27 (22.3%)	38 (31.4%)	42 (34.7%)	14 (11.6%)	121 (100%)	0.008

a: Pearson X^2 Test.

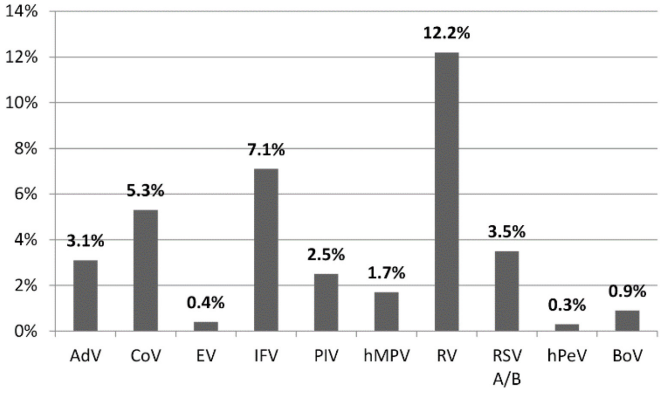
they developed respiratory symptoms along with other accompanying diseases.

It was observed that 914 (57.4%) of the samples were positive for any respiratory virus. The highest positivity was detected in the case of RV (24.6%) and influenza A viruses (12.1%); the lowest positivity was detected in the case of BoV (2.6%). The positivity rates of the patients according to their age groups are given in Figure 1. The distribution of viral agents by age groups is given in Table 1. The rates of CoV, hAdV, RSV A/B, hPIV, hMPV-A/B, hPeV, EV, and influenza B viruses were 9.9%, 8%, 7.7%, 4.8%, 3.3%, 3.1%, 3%, and 2.8%, respectively. In 37.8% (305/806) of all positive patients, more than one viral agent was detected. The distribution and rates of infection by these agents are listed in Table 2. Among the samples with more than one agent, the most common one is RV (13%), followed by Influenza Virus (7.8%). Co-positivity of rhinovirus and influenza A virus was detected in 7.9% (72/914) of the positive samples. In samples where more than three agents were present, those with amplification curves after the 35th cycle were evaluated as a cross-reaction and were interpreted as false positive. The percentages of the samples where a single viral agent was identified are presented in Figure 2. When the distribution of viral agents by months was examined; the highest positivity rates were observed in December (18%) and January (22.7%) and the lowest rate was in June (1.7%) (Figure 3). Influenza A and B viruses were seen mostly in winter, between November and May. RV, on the other hand, was mostly detected in autumn, winter and spring months. RSV A/B was detected throughout the year except in summer and had higher prevalence in winter. The distribution of the rates of viral agents by years is presented in Figure 4.

Discussion

The timely identification of viral respiratory pathogens is especially important in early diagnosis and clinical decision making. Molecular methods such as

Figure 2. Rates of single infections.



IFV: Influenza virus; RSV A/B: Respiratory syncytial virus A/B; RV: Rhinovirus; PIV: Parainfluenza virus; hMPV: Human metapneumovirus; CoV: Coronavirus; AdV: Adenovirus; BoV: Bocavirus; hPeV: Human parechovirus; EV: Enterovirus.

Table 2. Distribution of single and co-infections.

Viral Agents*	IFV	RSV	RV	PIV	hMPV	CoV	AdV	BoV	PeV	EV
IFV	113	6	95	5	9	21	13	9	3	3
RSV		55	20	1	0	7	12	3	36	6
RV			194	14	8	34	24	11	7	24
PIV				40	2	6	9	4	1	1
hMPV					27	7	6	2	3	1
CoV						81	8	4	2	6
AdV							50	5	8	13
BoV								13	2	2
PeV									4	2
EV										7
Dual infections	104	42	151	25	19	52	51	14	25	21
Triple infections	21	26	46	11	7	24	26	14	20	19
Total	238	123	391	76	53	157	127	41	49	47

*IFV: Influenza virus; RSV: Respiratory syncytial virus; RV: Rhinovirus; PIV: Parainfluenza virus; hMPV: Human metapneumovirus; CoV: Coronavirus; AdV: Adenovirus; BoV: Bocavirus; PeV: Parechovirus; EV: Enterovirus.

PCR and RT-PCR are sensitive and specific methods for detecting viruses. The multiplex PCR method requires less workforce and enables the detection of many pathogens at the same time [3]. Thus, detailed epidemiological data on viral respiratory pathogens could be obtained. In this study, 3-years data of viral respiratory agents detected in a university hospital were evaluated retrospectively. One or more viral agents were detected in 57% of the respiratory samples sent to the laboratory with suspicion of viral respiratory infections. The most common viral pathogen was RV (24.6%), followed by influenza A virus (12.1%) and CoV (9.9%). Along with the CoV, RV are also responsible for most of the upper respiratory tract infections and a significant proportion of lower respiratory tract infections. Many studies have shown that RV combined with RSV in children and influenza in the elderly are among the leading causes of pneumonia, bronchiolitis and other serious respiratory diseases [7,8]. In our study, RSV A/B was found as 7.7% of the samples tested. According to the age distribution, it was observed that children in the 0-3 years group were infected with RSV at a higher rate (44.6%) ($p < 0.001$). We observed that the rate of influenza virus infection in the same age group was lower (12%) compared to other age groups ($p < 0.001$) and was most frequent in the 19-65 age group (34.5%). According to Centers for Disease Control and Prevention (CDC), during the 2017-2018 influenza season, 1 out of every 217 Americans, aged more than 65 years, was hospitalized because of influenza infection [9]. A molecular epidemiologic study of respiratory viruses among young children in Malaysia reported that EV/RV and RSV constituted most of the viral respiratory infections [10]. Wang *et al*. reported that the total detection rate of respiratory viruses decreased with rising age in children in China. Higher rates for RSV and hPIV were detected in younger children and influenza virus rates were higher with increasing age [11]. Other viral factors are not dominant in any age group; however, RSV, hPIV, RV and AdV were found to be lower in the age group above 65 compared to other age groups (11-15%). As a result, a statistically significant relationship was found between the age groups of RSV, influenza, parainfluenza, RV

Figure 3. Monthly distribution of some viral agents.

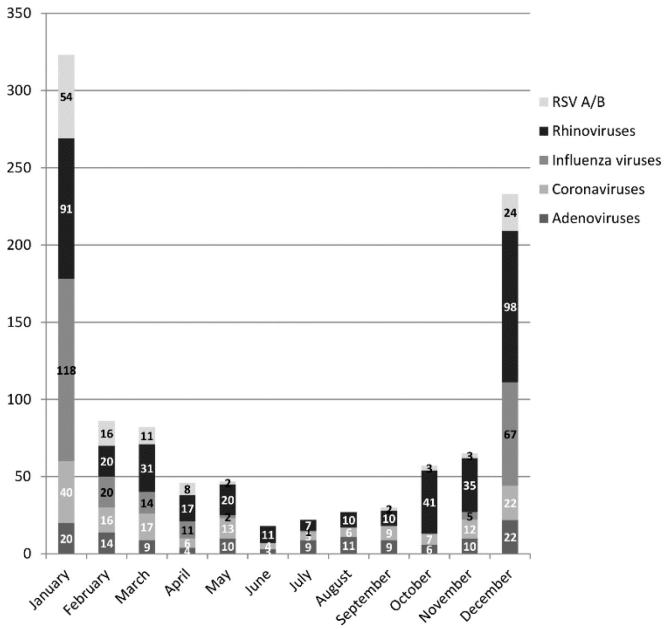
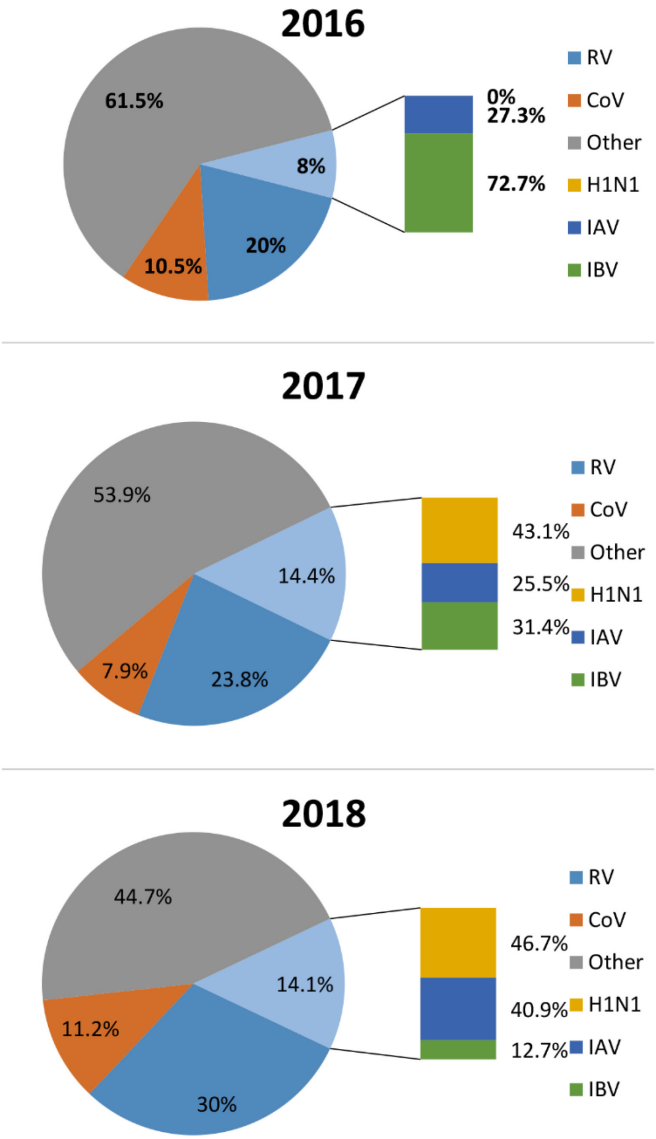


Figure 4. Three-year distribution of viral agents.



RV: Rhinovirus; CoV: Coronavirus; H1N1: Influenza A virus H1N1; IAV: Influenza A virus; IBV: Influenza B virus.

and AdV (Table 1). Studies conducted in Turkey showed that RSV was detected at higher rates in infants under 2 years of age [12,13]. The study by Çiçek *et al*. reported that influenza viruses were the highest in adult patients; and RSV was the highest in pediatric patients [13]. Influenza surveillance data in Turkey indicated that influenza viruses are detected at higher rates in adult patients [14].

The recent widespread use of molecular methods (such as multiplex RT-PCR) has helped to identify an increased number of related viral infections [15]. In the present study, multiple causative infections were observed in 37.8% of all positive patients. In previous studies, the rates of multiple causative infections were in the range of 5-62%; it was reported that RSV was found most frequently in these infections and AdV, IAVand BoV were found most frequently along with RSV [16]. In the present study, RV + IFV and RSV + PeV coexistence were observed mostly in multiple agent infections. Rhinoviruses and enteroviruses are in the Picornaviridae family and are genetically closely related [17]. Previous studies have reported that some samples detected RV using multiplex PCR method and detected EV through sequence analysis [18,19]. Thus, there may be a cross reaction between RV and EV. According to the results of a multi-center study conducted in Europe, it has been reported in some tests that EV and PeVs gave positive results with RV [20]. In the present study, EV and PeVs were detected in co-infections more frequently than in single infections.

IFV and RV were detected in another coexistence study [21]. However, although the rate of hospitalization in intensive care unit (ICU) is higher in co-infected patients, it was reported that there were no statistically significant differences between single infections and coinfections in patients in terms of their severity [22].

The seasonal distribution of viral pathogens detected in the present study was found to be similar to other studies in Turkey and the rest of the world [4,13,14,18,23]. While positive results are found at low rates in summer, this rate increased significantly in winter. Specifically, the occurrence of IFV, RV, RSV and CoV were seasonally variable and peaked in December and January. Price *et al*. analyzed the seasonality of respiratory viruses between 2009 and 2015 in Scotland. They reported that AdV and RV are present throughout the year and RV has a major peak around October-November. RSV and IAV have the largest seasonal peaks, appearing in November-December and December-January respectively [23]. In the present study RV, IFV and RSV had major peaks and CoV and AdV had minor peaks in December and January, respectively.

In the temperate Northern Hemisphere, the influenza season peaks from December to February [24]. Turkey's National Influenza Center defines the period from October to May as the influenza season, according to the findings of surveillance data [25]. In the present study, IFV positivity was detected between November and May and we observed that the highest rates were in December and January. According to the 2-year (2013-2014) annual sentinel surveillance results of Mese *et al*. in Turkey, IFV peaked in December and January [14]. In the present study, RV positivity (24.6%) was seen throughout the year, but it was found at higher rates in winter and spring, mostly in December and January. In the study by Demirkan *et al*., RV infections were reported most frequently between September and January and the highest rate was observed in November [18].

RSV is a frequent viral agent responsible for 43-74% of bronchiolitis cases [15]. In the present study, it was found in 7.7% of the samples and was most frequently encountered in December and January. In previous studies in Turkey, it has been reported that RSV rates are in the range of 5-22% [13,26,27]. Coronaviruses cause infections throughout the year, but they have the highest incidence in winter [4]. Coronavirus infections were detected in our study at a rate of 10% all year round and were most common in December-January.

While H1N1 was not detected in the present study in 2016, approximately half of the influenza cases were caused by the H1N1 virus in 2017 and 2018. The rate of occurrence of influenza A viruses increased year by year and the rates of influenza B viruses decreased. The proportion of RV has increased from 20% to 30% in three years (Figure 4). There was no significant change in the rates of CoV. Our results are similar to that reported in the influenza surveillance reports of the General Directorate of Public Health [25].

Conclusions

In conclusion, a viral respiratory tract pathogen was detected by the 'multiplex real-time PCR' method in 57% of the samples sent to Molecular Microbiology laboratory. It was observed that the RV were the most prevalent, followed by IFV. The co-positivity of RV and IFV are remarkable. RSV rates were found higher in the 0-3 years age group compared to the other age groups. IFV, RV, CoV, RSV and AdV showed seasonal variation and were found to be more prevalent in the winter months. Multiplex RT-PCR is a fast and useful

method for identifying many viral respiratory agents simultaneously in routine diagnostic microbiology laboratories, especially in high-risk patient groups. Using this method, we evaluated samples collected over a three-year period at our hospital and the epidemiological data was communicated with the clinicians, thereby preventing incorrect antibiotic usage.

Authors' Contributions

G.B. designed the study; A.T. and M.D. collected the samples and patients' data; A.A.K. performed experiments and analyzed data; A.A.K. and S.S. wrote the manuscript; G.B. and A.B gave scientific support and conceptual advice.

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Conflict of interests: No conflict of interests is declared.