Viral and Atypical Bacterial Respiratory Infections in a University Teaching Hospital

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SUMMARY: Respiratory viral and atypical bacterial agents lead to infections in a large spectrum, from mild symptoms to respiratory failure. In the present study, we aimed to detect multiple viral and bacterial agents in the respiratory samples of inpatients by real-time polymerase chain reaction (RT-PCR). Nasopharyngeal swabs and broncho-alveolar lavage samples from inpatients with respiratory infection symptoms at the Uludag University Hospital between December 1, 2015 and March 31, 2018 were investigated. DNA/RNA was extracted using the EZ1 Virus Mini Kit v2.0 (Qiagen, Belgium) with the EZ1 extraction device (Qiagen, Belgium). The R-GENE® RT-PCR (Biomerioux, France) kit was used to detect influenza A, influenza B, respiratory syncytial virus (RSV), human metapneumovirus, rhinovirus/enterovirus (RV/EV), adenovirus, human bocavirus (hBoV), corona virus, parainfluenza virus, Chlamydia pneumoniae/Mycoplasma pneumoniae, and Legionella pneumophila in Rotor-Gene Q (Qiagen, Belgium). Patients were aged between 0 and 90 years. Overall, 177 (56.9%) patients were men and 134 (43.1%) were women. A total of 311 samples were analyzed, of which 214 (68.8%) were positive. In total, 360 agents, including 338 viruses and 22 bacteria, were detected. The commonest agents were influenza A+B (n = 65, 18.1%), hBoV (n = 64, 17.8%), RV/EV (n = 56, 15.6%), and RSV (n = 47, 13.1%). Rapid diagnosis of viral infections by RT-PCR is important for the specific treatment of patients.

INTRODUCTION

Acute respiratory infections are major causes of morbidity and mortality (1,2). Viruses are the major cause of respiratory infections. The commonest viral agents are influenza, parainfluenza, and respiratory syncytial virus (RSV). Vaccination against bacteria also leads to increased prominence of viral causes of these illnesses (1,3). Respiratory viruses and bacteria lead to infections in a large spectrum of people, from mild symptoms to pneumonias and respiratory failure. Acute respiratory failure is potentially fatal especially in patients with malignant diseases and those that are immunosuppressed. Mortality rates of respiratory infections have increased up to 70%, especially in patients with malignancy (4–8).

Difficulties in the diagnosis of viral agents have led clinicians to prescribe the use of antibiotics, resulting in antibiotic abuse, increased costs, and waste of resources (2,9). Although the investigation of viral respiratory pathogens can be done by viral cell culture, ELISA or serological tests, these tests are not routinely used in clinical laboratories due to the long and labor-intensive tests.

The polymerase chain reaction (PCR) is highly reliable, specific and sensitive. Recently, multiplex PCR assays that are used to identify multiple viral agents with a single test have been described (10–13). Therefore, multiplex PCR tests are commonly used for the diagnosis of viral and bacterial infections (1,2,14).

In the current retrospective study, we aimed to detect multiple viral and bacterial agents in the respiratory samples of inpatients by a commercial real-time PCR assay.

MATERIALS AND METHODS

Patients: Bursa Uludag University Hospital is a teaching hospital with 1,000 beds which is located in Bursa city, at the northwest part of Turkey. Our hospital is a referral center for about 5 million people. In the present study, inpatients with respiratory symptoms, including nasal congestion, rhinorrhea, nasal discharge, sneezing, sore throat and cough, were tested for respiratory viruses and atypical bacterial panels between December 2015 and March 2018 and their results were investigated retrospectively. The patients were divided into 5 groups according to their ages. Groups 1, 2, 3, 4, and 5 included patients aged 0–1, 2–5, 6–18, 19–65, and older than 66 years, respectively.

Samples: Respiratory samples of patients’ (nasopharyngeal swabs or bronchoalveolar lavage [BAL] samples) between December 1, 2015 and March 31, 2018 were included in the study. Nasopharyngeal swab samples were taken by FLOQ Swabs (Copan, Brescia, Italy) and transported to the laboratory by a special transport medium (UTM-RT, Copan) and stored at −80°C. The samples were analyzed within 5 days. Fiberoptic bronchoscopy was performed according
to the American Thoracic Society clinical practice guidelines for obtaining BAL (15). BAL specimens were obtained according to computed tomography images.

The BAL samples were taken from the sterile physiologic saline at room temperature by applying to peripheral bronchiole and alveoli in 20 mL aliquots (16). Each aliquot was injected with negative pressure to withdraw at least 60% of the volume delivered. At least 1 mL of the sample was added to the transport medium (UTM-RT, Copan) and stored at −80°C. The samples were analyzed within 5 days.

Nucleic acid extraction and real-time PCR (RT-PCR): Nucleic acid extraction was performed using the EZ1 Virus Mini kit v2.0 (QIAGEN, Antwerp, Belgium) kit was used for the manufacturer’s instructions.

Respiratory Multi-Well System MWS R-GENE (BioMerieux, Marcy l’Etoile, France) kit was used for Nucleic acid extraction and real-time PCR (RT-PCR): Nucleic acid extraction was performed using the EZ1 Virus Mini kit v2.0 (QIAGEN, Antwerp, Belgium) with the EZ1 extraction device (QIAGEN) according to the manufacturer’s instructions.

Respiratory Multi-Well System MWS R-GENE (BioMerieux, Marcy l’Etoile, France) kit was used for RT-PCR, in Rotor-Gene Q (Qiagen). The kit includes influenza A, influenza B, RV (A,B,C) (A,B,C,D), adenovirus (A,B,C,D,E,F,G), and 17 C. pneumoniae/M. pneumoniae and 5 L. pneumophila. Distribution of viruses among age groups of commonest viruses are listed in Table 1. There were 42 samples from adults with a positivity rate of 57.1%. The positivity rate of children was 71.0%. The difference between adults and children was not significant (p = 0.11).

We compared the distributions of positives versus negatives from Table 1 across the age groups using a chi-square test evaluated with SPSS. Statistical analyses of RSV distribution showed a significant difference (p < 0.05) between Group 1 and other groups.

Analyses of Influenza A+B showed statistical significance (p = 0.001) between Group 2 vs Group 1. No difference was detected between other groups. These results indicate that Influenza A+B is predominant in children between 2–5 years. There was no statistically significant difference between other viruses and bacteria among age groups. Additionally, no significant difference was detected among sex, viruses, and bacteria.

Most of the respiratory infections were seen in winter (Table 2). The coldest months of the year are January and February followed by December and March. Most of the samples were sent in these 4 months and most of the positive results were detected between December and March (Fig. 1). The number of positive results from samples were evaluated. However, no significant difference of infection rate among age groups were observed.

There were 9 BAL samples with 3 positive samples. Positive samples had only one virus in each sample; 2 RV/EV and 1 hBoV (33%).

The commonest co-infection included hBoV. hBoV infections were observed in 54 co-infections, and the other co-infecting viruses besides hBoVs were influenza A (16 cases), adenovirus (15 cases), and RSV (14 cases).

Table 1. Distribution of most common viruses according to age groups

| Age | No of samples n (%) | Inf A+B n (%) | hBoV n (%) | RV/EV n (%) | RSV n (%) |
|-----|---------------------|--------------|------------|-------------|----------|
| 0–1 | 95 (30.6)           | 11 (16.9)    | 24 (37.5)  | 15 (26.8)   | 31 (66.0) |
| 2–5 | 90 (28.9)           | 28 (43.1)    | 18 (28.1)  | 21 (37.5)   | 7 (14.9)  |
| 6–18| 84 (27.0)           | 19 (29.2)    | 13 (20.3)  | 14 (25.0)   | 6 (12.8)  |
| 19–65| 29 (9.3)            | 4 (6.2)      | 5 (7.8)    | 5 (8.9)     | 2 (4.2)   |
| ≥66 | 13 (4.2)            | 3 (4.6)      | 4 (6.3)    | 1 (1.8)     | 1 (2.1)   |
| Total| 311                 | 65           | 64         | 56          | 47        |

InfA, influenza A; InfB, influenza B; hBoV, human bocavirus; RV/EV, rhinovirus/enterovirus; RSV, respiratory syncytial virus; No of samples, Number of samples.
DISCUSSION

To the best of our knowledge, this is the first study that reports findings of respiratory viruses in Bursa. We detected a high positivity rate (68.8%) for respiratory infections, which is higher than that reported by Nyiru et al. (1) and Turner et al. (17). This high rate is due to a strict selection of patients’ sampling for respiratory infections and also due to the age of the patient group, as most of the patients were from pediatric clinics which have a higher rate of detection than adults.

The commonest viruses in our study were influenza A+B, hBoV, RV/EV, and RSV. Influenza viruses are the major causes of both upper and lower respiratory infections (17,18). We found that the influenza A virus infections’ peak is in January while influenza B has a peak in March. Influenza A distribution is concordant with the findings of the Turkish Ministry of Health’s Weekly Influenza Report (19). In the northern hemisphere, seasonal influenza A+B has a peak in January, during the influenza season between December and March (20). Influenza A has a peak in January in the northern hemisphere which is similar to our results, however, influenza B’s peak in our study was in March while the peak in the northern hemisphere was in January and the peak in our county was in February (19,20). This difference may be due to the low number of Influenza B cases in our study.

Furthermore, we found that hBoVs were very common in our patients. Additionally, we found most of the
hBoVs (54/64, 84%) as part of co-infections. Since the discovery of hBoV in 2005, it has been associated with upper and lower respiratory infections and gastroenteritis globally (21,22). There are seroepidemiologic studies demonstrating that the seropositivity rate for hBoV increases with age and reaches 100% by 6 years (23,24). Although hBoV is usually seen in co-infections, but it can be seen as mono-infection and sometimes it can be detected in asymptomatic children (25,26). These data demonstrate that our results about hBoV are concordant with the literature. However, to understand whether the detected hBoV is pathogenic or not, viral load (which could not be assessed in the present study) should be monitored.

Our kit detected RV or EV as RV/EV in one test. The positive result for RV/EV meant that the test was positive for RV/EV. Rhinoviruses cause common colds and are implicated in lower respiratory tract infections. Although the RV is a respiratory pathogen it has also been detected in up to 44% of asymptomatic cases, making the interpretation, and clinical relevance of a positive RV result in children unclear (27–29). Enterovirus D68 has similar characteristics to RV infections, and it is also associated with more severe disease (30,31). Rhinovirus/EV was the 3rd common pathogen in our study which is concordant with the findings of Turkish Ministry of Health’s Weekly Influenza Report, which ranked rhinovirus as the 3rd commonest virus (19). In the present study, the RV/EV distribution is from October to March which is longer than that of influenza and similar to the results of our country (19).

RSV is a major cause of respiratory infections in children younger than 5 years (32). The RSV infection peak was seen in January, which is similar to the findings of Viguira et al. (32). A significant proportion of RSV infections are in the first year of life. We found 66 % of RSV cases were below the age one and this finding is concordant with other studies (1,17,32). RSV infection is especially important in preterm infants, bronchopulmonary dysplasia, congenital heart disease, Down’s syndrome, and neuromuscular diseases (33,34). In Turkey, RSV was found as the major agent for severe acute respiratory infections, having a peak in January similar to our results (19).

Viral interference is a phenomenon that indicates that a virus-infected cell is inhibited from being infected by another virus (35). In viral accommodation, more than one virus infects a cell at the same time. If viruses infect the cell at the same time it is called co-infection and when viruses infect the cell one after the other it is defined as superinfection (35,36). In our study, more than 2 viruses were detected in 93 samples. Morikawa et al. (37) investigated 16 different viruses and found that 31% of the samples showed more than one virus and detected 5 different viruses in a patient. Therefore, when a test that includes a large number of viruses and bacteria is used, the possibility of detecting co-infections increases.

Palivizumab is used for the prophylaxis of RSV (32). Most of the RSV (66%) cases were in the 0–1 age group. Patients in this age group are not able to move, and they are always in the same hospital room or pediatric intensive care unit. Nosocomial infections due to a variety of microorganisms affects 0–1 age group (38–40). Hence, palivizumab has an important role in cases of an RSV infection for the prophylaxis of other patients in the same unit. Influenza A and B, C. pneumoniae, L. pneumophila have specific anti-viral or antibacterial treatment. In our study, we detected 65 Influenza A and B, 17 C. pneumoniae/M. pneumoniae and 5 L. pneumophila. This means that 24.2% (87/360) of respiratory infections in our study are suitable for specific treatment. Therefore, inpatients with respiratory infection symptoms should be tested for viruses and bacteria, especially with RT-PCR for rapid results (1,17,41). Specific antiviral or antibacterial therapy should be initiated in patients according to the results.

In conclusion, viral respiratory infections are usually seen in the pediatric-aged inpatients in our hospital. The commonest viruses are Influenza A and B, hBoV, RV/EV, and RSV. Respiratory infections are usually seen from December to March. During this period clinicians should be aware of viral respiratory infections. Rapid diagnosis of viral infections by RT-PCR is important for the specific treatment.

**Conflict of interest** None to declare.

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