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Etiology, Seasonality, and Clinical Characterization of Viral Respiratory Infections Among Hospitalized Children in Beirut, Lebanon

Mayda Finianos,[1] Randi Issa,[1] Martin D. Curran,[2] Claude Afif,[3] Maryam Rajab,[4] Jihad Irani,[3] Noha Hakimeh,[3] Amal Naous,[4] Marie-Joelle Hajj,[3] Pierre Hajj,[3] Tamima El Jisr,[5] and Mira El Chaar[1]*

[1]*Faculty of Health Sciences, University of Balamand, Beirut, Lebanon*
[2]*Public Health England Clinical Microbiology Laboratory, Addenbrooke's Hospital, Cambridge, United Kingdom*
[3]*Faculty of Medicine, University of Balamand, Saint Georges University Medical Centre, Beirut, Lebanon*
[4]*Department of Pediatrics, Makassed General Hospital, Beirut, Lebanon*
[5]*Laboratory Medicine, Makassed General Hospital, Beirut, Lebanon*

Acute respiratory tract viral infections occur worldwide and are one of the major global burdens of diseases in children. The aim of this study was to determine the viral etiology of respiratory infections in hospitalized children, to understand the viral seasonality in a major Lebanese hospital, and to correlate disease severity and the presence of virus. Over a 1-year period, nasal and throat swabs were collected from 236 pediatric patients, aged 16-year old or less and hospitalized for acute respiratory illness. Samples collected were tested for the presence of 17 respiratory viruses using multiplex real-time RT-PCR. Pathogens were identified in 165 children (70%) and were frequently observed during fall and winter seasons. Co-infection was found in 37% of positive samples. The most frequently detected pathogens were human Rhinovirus (hRV, 23%), Respiratory Syncytial Virus (RSV, 19%), human Bocavirus (hBov, 15%), human Metapneumovirus (hMPV, 10%), and human Adenovirus (hAdV, 10%). A total of 48% of children were diagnosed with bronchiolitis and 25% with pneumonia. While bronchiolitis was often caused by RSV single virus infection and hAdV/hBoV coinfection, pneumonia was significantly associated with hBoV and HP1V1 infections. No significant correlation was observed between a single viral etiology infection and a specific clinical symptom. This study provides relevant facts on the circulatory pattern of respiratory viruses in Lebanon and the importance of using PCR as a useful tool for virus detection. Early diagnosis at the initial time of hospitalization may reduce the spread of the viruses in pediatric units. ***J. Med. Virol. 88: 1874–1881, 2016.*** © 2016 Wiley Periodicals, Inc.

KEY WORDS: epidemiology; respiratory viruses; multiplex PCR; hospitalized children; Lebanon

INTRODUCTION

Viral infections of the respiratory tract are the most common cause of diseases and mortality in children under 5-year old [Liu et al., 2012]. Infants are more vulnerable to respiratory viral infections with approximately six to eight infections per year [Tregoning and Schwarze, 2010]. Most of these infections are confined to the upper respiratory tract (URT) leading to symptoms of common cold, coryza, and cough and are often accompanied by fever with fatigue and loss of appetite in some cases. Approximately, one third of infants with respiratory viral infections develop lower respiratory tract (LRT) symptoms that may lead to bronchiolitis, pneumonia, and severe respiratory distress [Tregoning and Schwarze, 2010].

Abbreviations: Flu A, Influenza A virus; Flu B, Influenza B virus; RSV, Respiratory Syncytial Virus; hMPV, Human Metapneumovirus; hRV, Human Rhinovirus; hEV, Human Enterovirus; hAdV, Human Adenovirus; HPIV, Human Parainfluenza viruses; CoV, Coronaviruses; hBoV, Human Bocavirus; URT, Human Bocavirus; LRT, Lower respiratory tract; MGH, Makassed General Hospital

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*Correspondence to: Mira El Chaar, Faculty of Health Sciences, University of Balamand, P.O. Box 166378, Ashrafieh, Beirut 1100-2807, Lebanon.
E-mail: mira.elchaar@balamand.edu.lb

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In Lebanon, there is a lack of epidemiological data on many respiratory viruses and their clinical impact. This is often related to the high cost or absence of sensitive assays that would identify respiratory viruses. The aim of the study was to determine the etiology of respiratory infections among pediatric patients admitted to a major Lebanese university hospital, to observe the association between the disease's symptoms and the identified virus and to understand the seasonality of circulating viruses.

MATERIALS AND METHODS

Specimen Collection

Makassed General Hospital (MGH) is a 200 beds hospital located in a medically underserved population and heavily populated area of Beirut. It serves nearly 15,000 inpatients per year that are mostly from middle to low socioeconomic status. This study was approved by the institutional review board of MGH and was conducted during a 1-year period, from October 2013 to September 2014.

Nasal and throat swabs were collected from hospitalized pediatric patients (≤16-year old) who presented with symptoms of acute respiratory infection. All the participants' guardians signed an informed consent form for participation in this study. Any patient readmitted to the hospital with similar symptoms in less than 1 month was excluded. A standardized questionnaire for each child was filled by the pediatrician. The questionnaire included variables such as age, gender, admission and discharge dates, history of illness, smoking history, co-morbidities, clinical symptoms, illness diagnoses, antibiotic therapy, and Influenza vaccination status. Both nasal and throat swabs from each patient were stored in one viral transport medium tube (MicroTest M4RT, Remel, Lenexa, KS) at −80° C prior to testing.

Nucleic Acid Extraction

Nucleic acids were extracted from 200 μl of VTM stored sample and eluted in 60 μl of elution buffer. High Pure Viral Nucleic Acid kit (Roche, Germany) was used according to the manufacturer's instructions. Internal control (bacteriophage MS2) was added (4,600 pfu per extraction) to all samples prior to extraction.

Multiplex PCR for Respiratory Viruses

Seventeen viral pathogens were included in the multiplex reverse transcription real time PCR assays. Seven panels were tested for the following viruses: Influenza A virus (Flu A; H1 and H3 subtypes), Flu B, Respiratory Syncytial Virus (RSV), human Metapneumovirus (hMPV), human Rhinovirus (hRV), human Enterovirus (hEV) human Adenovirus (hAdV), human Parainfluenza viruses (HPIV 1–4), group 1 Coronaviruses (CoV-229E and CoV-NL63), group 2 Coronaviruses (CoV-OC43, and CoV-HKU1), and human Bocavirus (hBoV). Primers-probes sets (Metabion, Planegg/steinkirchen, Germany) have been detailed elsewhere [Clark et al., 2014, 2015]. Positive controls for the respiratory viruses were kindly donated by Addenbrooke's hospital clinical microbiology laboratory, Cambridge, UK. The triplex or duplex PCR reactions included 2× reaction mix containing 0.2 mM of dNTP, 2 to 6 mM MgSO4, 20 μM of forward and reverse primers, 10 μM of fluorogenic probes, 1 μl of superscript III RT platinum taq (Invitrogen, Carlsbad, CA), and 5 μl of purified nucleic acid. The reaction was initiated by a reverse transcription step at 50°C for 30 min followed by an amplification cycle with the following conditions: a cycle of 95° C for 15 min, then 45 cycles of 15 sec at 95° C, and 1 min at 60°C with subsequent acquiring of the appropriate fluorescence reading.

Statistical Analysis

Data analysis was performed using SPSS (version 20.0). Simple descriptive statistics were used to calculate the mean, median, and standard deviation for the quantitative data and proportions were used for the qualitative data. Continuous variables, which included mean age, length of hospitalization, and duration of symptoms were analyzed by Student's *t*-test or Mann–Whitney U test for parametric and nonparametric data, respectively. Categorical variables, such as seasonality, respiratory symptoms, comorbidity and clinical diagnosis were evaluated by chi-squared or Fisher's exact test when appropriate. All *P*-values less than 0.05 were considered statistically significant.

RESULTS

Epidemiological Data

A total of 236 children were enrolled in this study, 135 males (57.2%) and 101 females (42.8%) with an age ranging between 4 days and 13.7-year old (median age of 1 year). The data collected from the questionnaires (Table I) showed that 24% (n = 56/236) of children were enrolled in a school or daycare and 64% (n = 152/236) had at least one smoker living in the same household. Seven percent of patients (n = 17/236) had comorbidities such as congenital diseases (n = 3), cerebral diseases (n = 5), gastrointestinal diseases (n = 1), G6PD deficiency (n = 3), immunodeficiency diseases (n = 3), thalassemia major (n = 1), and renal disease (n = 1). Twenty-three percent (n = 54/236) had asthma and 5% (n = 11/236) had allergic diseases (Table I). The median duration of symptoms among infected children was 4 days and the length of their hospitalization ranged between 3 and 7 days (median: 5 days).

Overall, antibiotics were prescribed to 33% of children (n = 77/236). Among all hospitalized children, 6% (n = 15/236) were vaccinated for influenza virus.

TABLE I. Demographic and Clinical Characteristics of Hospitalized Children

	Values (%)[a]	Number of symptomatic patients positive for any viral infection (%)[b]
Exposed to smoking in the house	152 (64.4)	104 (68)
Attending a day care	56 (23.7)	37 (66)
Comorbidities	17 (7.2)	12 (71)
History of pulmonary disorders		
Suffering from asthma	54 (22.8)	38 (70)
Suffering from allergies	11 (4.6)	9 (82)
Respirator distress syndrome	3 (1.2)	2 (67)
Other pulmonary conditions	6 (2.5)	3 (50)
Clinical symptoms		
Fever > 38	144 (61)	105 (73)
Headache	15 (6.3)	15 (100)
Chills	26 (11)	21 (81)
Myalgia	24 (10)	22 (92)
Runny nose/ sneezing	180 (76.2)	128 (71)
Nasal congestion	161 (68.2)	112 (70)
Pharyngitis	25 (10.5)	16 (64)
Conjunctivitis	38 (16.1)	32 (84)
Tender glands in neck	9 (3.8)	6 (67)
Hoarseness	23 (9.7)	19 (83)
Dry cough	125 (53)	86 (69)
Productive cough	116 (49)	82 (71)
High respiratory rate	152 (64.4)	109 (72)
Shortness of breath	133 (56.3)	95 (71)
Pleuritic chest pain	17 (7.2)	12 (71)
Wheezes	100 (42.3)	72 (72)
Croup	9 (3.8)	5 (56)
Presence of cyanosis	13 (5.5)	7 (54)
Septic shock	4 (1.6)	2 (50)
Number of patient with High-sensitivity CRP > 3 mg/L	52 (22)	42 (81)
Admission to ICU	24 (10.1)	21 (88)
Oxygen need	26 (11)	20 (77)
Intubation	7 (3)	6 (86)
Diagnosis upon discharge		
Bronchiolitis	116 (49)	80 (69)
Pneumonia	61 (26)	42 (69)
Athma exacerbation	38 (16)	25 (66)
Other pulmonary conditions	12 (5)	7 (58)
Otitis media	9 (4)	6 (67)
Antibiotic	77 (32.6)	54 (70)
Influenza vaccine	15 (6.3)	13 (87)

The value represents the total number of patients positive for a specific symptom.
[a]The percentage was calculated by dividing the value over the total number of patients tested (n = 236).
[b]The percentage viral positivity of symptomatic patients was calculated by dividing the total number of patients positive for the virus and symptom over the total number of patients positive for the symptom.

Etiology and Seasonality of Respiratory Viruses

Overall, 165 out of 236 samples (70%) collected at MGH were positive for respiratory viruses. Among the total positive samples, 63% (n = 104/165) were found to be positive for at least one respiratory virus and 37% (n = 61/165) contained two or more viruses. Comparisons between age groups and specific virus infection showed that RSV was detected in 26% (n = 28/108) of the 108 children aged less than 1-year old and in 13% (n = 17/128) of the 128 children aged above 1-year old (P-value = 0.02). Moreover, when analyzing 26 patients infected with a single RSV infection, 18 (69%) were aged below one and 8 (31%) were aged one and above ($P = 0.017$). The median age of infants, aged less than one year, infected with RSV was 6.5 months. Among 76 children infected with a single non-RSV infection, 44 (58%) were 1-year old and above and 32 (42%) were aged below 1 year ($P > 0.05$).

Positive detection rate for respiratory viruses each month varied between 40% and 100% (median of 71%) (Fig. 1). Despite the low number of total samples collected in the summer (n = 20), all were positive for viral infections.

In this study, hRV (n = 54/236, 23%), RSV (n = 45/236, 19%), hBoV (n = 36/236, 15%), hMPV (n = 23/236, 10%), and hAdV (n = 24/236, 10%) were the most prevalent (Fig. 2). The rest of the respiratory viruses were present at a percentage lower than 5%. Flu A infected 12 patients (n = 12/236, 5%); among those two were positive for H1 and 10 for H3.

In this study, single infection was seen in children infected with Flu A (n = 10/12, 83%) and RSV (n = 26/45, 48%), however, a high number of coinfection was

observed for hAdV (n = 21/24, 86%), hRV (32/54, 59%), and hBoV (28/36, 78%) (Table II). From the total 61 coinfected individuals, hBoV was observed more frequently with AdV (n = 17/61, 28%) or hRV (n = 13/61, 21%). Only two children were infected with four or five viruses, respectively.

Detection of any viral agent was frequently observed during fall and winter seasons (Fig. 3) from October 2013 to March 2014 with the highest number of positivity seen in December (n = 29). The seasonal distribution varied according to each virus; RSV (73% of RSV A (n = 33/45) and 27% (n = 12/45) of RSV B) was circulating in the fall and winter with peak activity in December and January. Flu, hMPV and CoV infections mainly occurred in the winter seasons. hBoV infection occurred in all seasons except spring but hAdV and hRV were distributed all year round. The latter three viruses were the most commonly observed respiratory viruses in the summer. Enterovirus detection had mostly winter and spring seasonality. HPIV1 and HPIV2 occurred in fall however, HPIV3 and HPIV4 occurred sporadically throughout the year (Fig. 3).

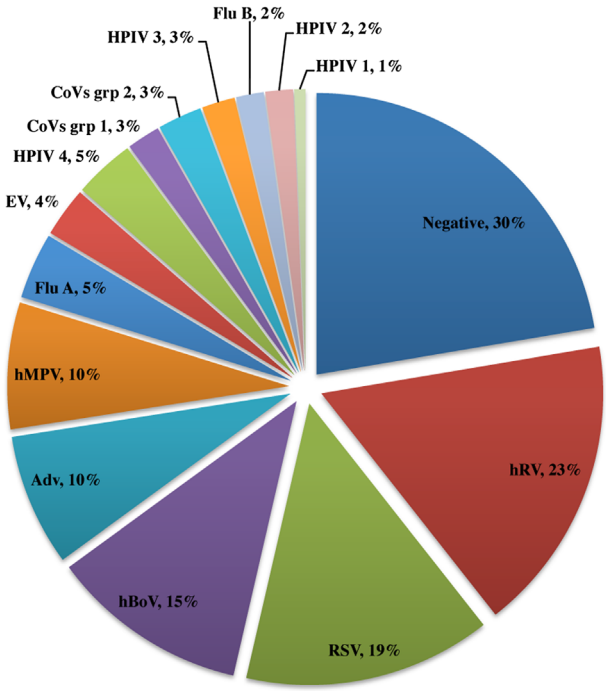

Fig. 2. Prevalence of respiratory viruses detected in hospitalized children during a 1-year period.

Association of the Patients' Clinical Symptoms With the Respiratory Viruses

All patients were assessed for their clinical symptoms upon sample collection, their discharge diagnosis, and antibiotics usage during their hospitalization. Most of the children admitted had fever (n = 144/236, 61%), runny nose (n = 180/236, 76%), nasal congestion (n = 161/236, 68%), dry cough (n = 125/236, 53%), productive cough (n = 116/236, 49%), high respiratory rate (n = 152/236, 64 %), and shortness of breath (n = 133/236, 56%). Other clinical signs were present at a lower percentage (Table I).

Respiratory viruses, whether present as single or multiple infections, exceeded 50% in symptomatic children (range: 50–100%). For instance, among children producing productive or nonproductive cough, 71% (n = 168/241) were positive for any viral infection (Table I).

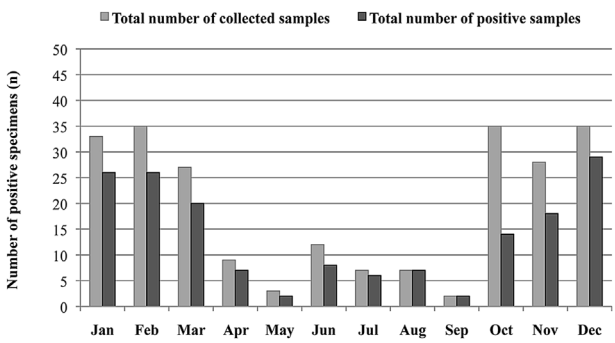

Fig. 1. Total number of collected and positive samples each month. Detection rates were highest in Fall and Winter season.

From the total 165 infected patients, 21 (12.7%) were admitted to ICU and 7 of them were intubated. A total of 80 children were diagnosed with bronchiolitis (n = 80/165, 48%), 42 (n = 42/165, 25%) with pneumonia, 25 (n = 25/165, 15 %) with asthma,and 6 (n = 6/165, 3.6%) with otitis media. RSV single infection (n = 20/45, 44%, $P < 0.01$), Flu A (n = 6/12, 50%, $P > 0.05$), and hAdV/hBoV coinfection (n = 9/24, 38%, $P < 0.02$) were often causing bronchiolitis. The latter clinical outcome was less diagnosed in patients infected with hRV single infection (n = 9/54, 17%, $P > 0.05$) (Table III).

Pneumonia was diagnosed in 25 patients infected with a single virus; RSV (n = 7/45, 16%, $P > 0.05$), Human Bocavirus (n = 4/36, 11%, $P <$ 0.05), hMPV (n = 5/23, 22%, $P > 0.05$), and HPIV1 (n = 2/2, 100%, $P < 0.05$) were the most common causative agents. Coinfection of RSV with hRV caused three cases of pneumonia (Table III). Asthma exacerbation was seen in patients infected with either a single CoV (n = 3/6, 50%, $P < 0.05$) or hBoV infection (n = 3/36, 8%, $P < 0.05$) (Table III).

Extensive analysis was performed to study the correlation between a single and multiple viral etiology infection with a specific clinical sign; data showed no significant associations (data not shown).

DISCUSSION

This study is the first to examine the viral etiology of acute respiratory illness in hospitalized children in Beirut, Lebanon over four seasons. We determined the

TABLE II. Total Number of Respiratory Viruses and Co-Infections Detected in Hospitalized Children

Viruses	Flu A	Flu B	CoV grp 1	CoV grp 2	hBoV	RSV	EV	hRV	hAdV	hMPV	HPIV 1	HPIV 2	HPIV 3	HPIV 4
Flu A	**12**	0	0	0	1	0	1	0	0	0	0	0	0	0
Flu B		**5**	0	0	0	0	0	0	0	2	0	0	0	0
CoV grp 1			**6**	0	1	1	0	0	1	0	0	0	0	0
CoV grp 2				**8**	2	4	1	0	1	2	0	0	0	0
hBoV					**36**	5	0	13	17	1	0	1	1	4
RSV						**45**	3	9	1	1	0	0	0	2
EV							**9**	2	0	0	0	0	0	0
hRV								**54**	11	5	0	2	1	2
Adv									**24**	0	0	0	3	1
hMPV										**23**	0	0	0	0
HPIV 1											**2**	0	0	0
HPIV 2												**5**	0	2
HPIV 3													**6**	1
HPIV 4														**11**
Viral Co-infection														
1 virus	10	3	4	2	8	26	4	22	3	12	2	1	3	4
2 viruses	2	2	1	3	13	12	3	20	10	11	0	3	2	4
3 viruses	0	0	1	2	13	6	2	11	9	0	0	1	0	2
4 viruses	0	0	0	1	1	1	0	0	1	0	0	0	0	0
5 viruses	0	0	0	0	1	0	0	1	1	0	0	0	1	1

The number in bold (vertically) indicates the total number of patients positive for the virus. Co-infections were commonly observed (seen horizontally on the table).
The lower section of the the table, when read vertically, represents the total number of patients positive for each virus as single or multiple viruses. Five types of viral co-infections were observed; one patient was infected with five virus (observed horizontally).

prevalence of 17 respiratory viruses in a population where there is a lack of information about the circulatory patterns of respiratory viruses: FluA, FluB, RSV, hMPV, AdV, hBoV, PIV1-4, hEV, hRV, CoV, and SARS. Of the total 236 samples collected from MGH, 70% of patients were infected by at least one virus; which is consistent with the high rate observed worldwide [Liu et al., 2014; Mengelle et al., 2014; Ouedraogo et al., 2014]. The positivity rate for any microbial agent may possibly be higher since undiagnosed infection could be positive for bacterial infections that were not included in the screening panel. Existing pathogens may include *Streptococcus pneumonia, Bordatella pertussis, mycoplasma penumoniae, Chlamydia peneumonia, and Legionella pneumophila* [File et al., 1998]. Thus, introducing these bacteria to the panel may improve the detection rate of bacterial single infection or bacterial and viral co-infections.

Most of the viral infections were observed in the winter and fall seasons. The viral prevalence in the summer was low; this can be associated to less number of symptomatic children seeking medical care during this season and to reduced aerosol transmission at high temperature for some viruses such as influenza and RSV [Lowen et al., 2007; Lowen and Steel, 2014; Paynter, 2015]. In our study, the prevalence of Flu A (5%) was higher than Flu B (2%), with the predominance of H3N2 (83%) in hospitalized children. Zaraket et al. [2014] studied virus prevalence in all age groups and previously reported that H3N2 virus predominated in Lebanon during the 2011–2012 seasons. However, a year before, both 2009 pandemic H1N1 and B viruses co-circulated with equal prevalence [Zaraket et al., 2014]. Despite the global predominance of a specific Flu subtype, circulating strains may vary in each influenza season.

The advantage of using a multiplex real time PCR assay in the diagnosis of respiratory viruses is to provide information on the presence of viruses and significance of co-infection. Our study demonstrated 37% of viral coinfection, which was higher than other reported studies where co-infection rate ranged between 14% and 31% [Shiley et al., 2010; Lekana-Douki et al., 2014]. In fact, hBoV was often associated with high rates of co-infections with AdV and hRV.

In keeping with previous studies, we detected respiratory viruses in 69% (n = 80/116) of patients diagnosed with Bronchiolitis and with pneumonia (n = 42/61) and 66% (n = 25/38) with asthma [Griffin et al., 2004]. RSV and hMPV were often causing lower respiratory tract infection. From all samples tested, we frequently detected RSV that infected infants less than 1-year old suggesting that these children may lack passively acquired immunity from their mother or the antibodies were not effective in preventing the infection. A comparable prevalence of RSV was previously reported in 2008 in the northern part of Lebanon (26.7%), in Turkey (20%), and in

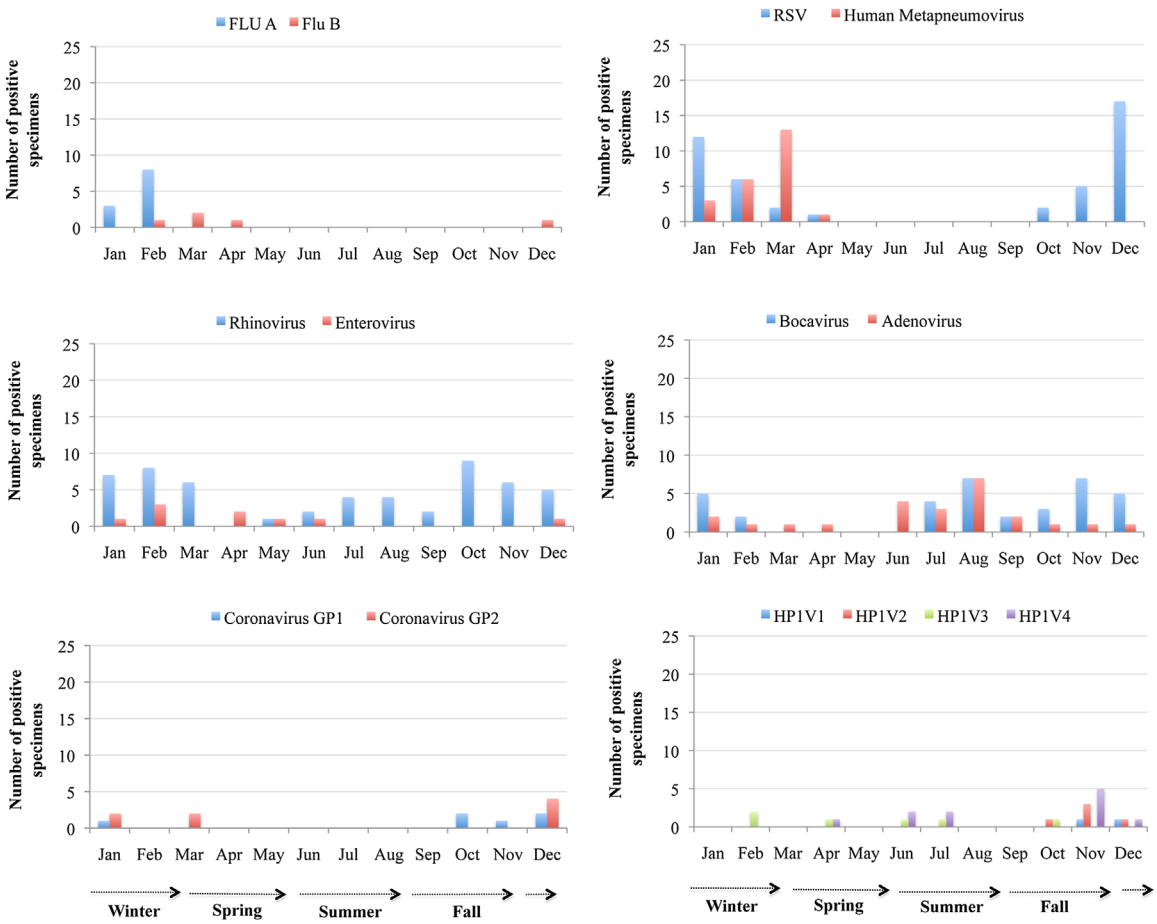

Fig. 3. Monthly distribution of respiratory viruses detected in 1-year period. The bars represent the total number of samples positive for the virus.

Jordan (34%) [Bdour, 2001; Kanra et al., 2005; Hamze et al., 2010].

Viruses such as hBoV, hRV, and RSV were frequently observed with other viral pathogens. Therefore, identifying the pathogenic role of these viruses on the disease severity of the patients is challenging. It was previously documented that viruses may be persistent in asymptomatic individuals who may be also shedding in the nasal secretion for 11 days, 3 weeks and 6 months in hRV, RSV, and hBoV infections, respectively [van Benten et al., 2003; von Linstow et al., 2008; Blessing et al., 2009; Peltola et al., 2013; Piedimonte and Perez, 2014]. The high rate of co-infections led us to explore the association between the presence of multiple viruses and severity of symptoms. According to our results and in accordance with previous studies, we found no differences in clinical severity between patients hospitalized with single infection and those with viral co-infection [Aberle et al., 2005; Paranhos-Baccala et al., 2008; Asner et al., 2014]. However, a previous study conducted by Semple et al. [2005] demonstrated that coinfection with both hMPV and RSV increase by 10-fold the risk of admission for intensive care unit and the use for mechanical ventilation. This hypothesis could not be applied in our study since only one child was infected with both viruses and was not admitted to ICU for ventilation.

This study has few limitations. We did not investigate the pathogens prevalence in asymptomatic children at the pediatric unit, which could have explained the silent role of a single or a co-infection of viruses in non-sick children. In addition, we could not address whether bacterial or other viral pathogens were found in all samples including the negatives ones. In our study, we used nasal and throat swabs instead of nasopharyngeal swab, which is a more invasive procedure with a higher sensitivity [Do et al., 2011]. This could also explain the negative results in 30% of the children.

In conclusion, our findings exemplify the epidemiology of respiratory viruses in a major hospital in Lebanon and their seasonality during one year period. We have identified 16 circulating viruses that were common causes of a single infection and co-infections in hospitalized children. The high percentage of positivity reflects the burden of respiratory viral infections, yet further studies are

TABLE III. Correlation Between the Presence of the Virus and the Clinical Diagnosis Observed in Infected Children

	Flu A (n = 12)	Flu B (n = 5)	CoV grp 1 (n = 6)	CoV grp 2 (n = 8)	hBoV (n = 36)	RSV (n = 45)	hEV (n = 9)	hRV (n = 54)	hAdV (n = 24)	hMPV (n = 23)	HPIV1 (n = 2)	HPIV2 (n = 5)	HPIV3 (n = 6)	HPIV4 (n = 11)
Single infection														
Bronchiolitis	6	1	0	1	1	**20**	1	9	1	3	0	0	2	3
Pneumonia	1	2	0	1	**4**	7	0	2	1	5	**2**	0	0	0
Asthma	1	0	**3**	0	**3**	0	2	3	0	1	0	0	0	1
Exacerbation														
Acute Otitis Media	1	0	0	0	0	0	0	1	1	1	0	0	0	0
Double infection														
Bronchiolitis	0	1	1	1	9	5	1	10	**9**	4	0	2	1	2
Pneumonia	0	0	0	0	1	5	2	6	1	2	0	0	1	0
Asthma	2	0	0	2	3	2	1	5	0	4	0	0	0	1
Exacerbation														
Acute Otitis Media	0	1	0	0	1	0	0	0	1	1	0	0	0	0
Triple infection														
Bronchiolitis	0	0	0	1	5	3	1	6	4	0	0	0	0	0
Pneumonia	0	0	1	1	6	3	1	3	3	0	0	1	0	2
Asthma	0	0	0	0	1	0	0	1	1	0	0	0	0	0
Exacerbation														
Acute Otitis Media	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Bold underlined numbers represent statistically significant data (P-value < 0.05).

needed to study the impact of bacterial and viral co-infection in these children and to discover new microorganisms that may play a role in cases with severe respiratory infection with unknown etiologies.

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