Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Viral Etiology of Acute Respiratory Infections in Pediatric Patients in Lebanon

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Competing interests: The authors declare no conflict of Interest.

Abstract. ***Background*: Acute respiratory infections (ARI) are the leading cause of death worldwide, especially among children. The majority of these infections in children are of viral etiology. In this study, we evaluated the incidence of viral ARI among children in Lebanon.**
***Patients and Methods*: Children presenting with symptoms of ARI were prospectively recruited between September 2009 to February 2012. Nasopharyngeal aspirates were obtained from patients and screened for 11 respiratory viruses using a multiplex Luminex-based PCR assay.**
***Results*: Two hundred twenty-one patients were recruited with a median age of 1 year (IQR: 0 - 5). Out of 221 patients, 116 (52.5%) were positive for at least one virus, the majority (103/116; 88.8%) of which were in children under 6-year of age. Overall, 188 viruses were detected. Rhinovirus (RhV) was the most common virus detected in 81 (69.8%) patients followed by coxsackie virus and echovirus (CVEV) which were detected as one target in the panel in 45 (38.8%), and parainfluenza viruses (PIV types: 1, 2, 3, 4) in 24 (20.7%) patients. Coinfection with more than one virus was detected in 49 (42.9%) patients. RhV and CVEV were the most common viruses associated with co-infections and higher risk of rhinorrhea.**
***Conclusions*: Viral pathogens account for at least half of the ARIs in Lebanon, with a high frequency of co-infections being detected.**

Keywords: Children Viral infections; Respiratory; Luminex; Molecular diagnosis.

Citation: Masoud K., Hanna-Wakim R., Zaraket H., Araj G., Matar G., Dbaibo G. Viral etiology of acute respiratory infections in pediatric patients in Lebanon. Mediterr J Hematol Infect Dis 2019, 11(1): e2019059, DOI: http://dx.doi.org/10.4084/MJHID.2019.059

Published: November 1, 2019 **Received: June 24, 2019** **Accepted: September 25, 2019**

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Introduction. Acute respiratory tract infections (ARIs) are among the most common reasons for primary care consultations.[1] The World Health Organization (WHO) ranks ARIs as the fourth major killer after cardiovascular diseases, general infections and parasitic diseases, and cancer.[2] ARIs cause 4 million death globally. The burden is especially high in children where ARIs are responsible for 11-22% of deaths.[3] ARIs can lead to severe complications requiring hospitalizations and can have fatal outcomes.

Viruses are the most common etiology of ARIs in children.[6,7] These include rhinovirus (RhV), respiratory

syncytial virus (RSV), influenza (IFN), parainfluenza virus (PIV), coronavirus (CoV), human metapneumovirus (hMPV), enteroviruses (EV), adenovirus (AdV), and human bocavirus (HBoV).[6,8,9] Each of these viruses poses a significant health burden. Nair et al. estimated that 111 500 deaths in children <5 years were attributable to influenza-associated lower respiratory tract infections (LRI) in 2008, the vast majority of which occurred in developing countries.[10] RSV was estimated to have caused 33.8 million LRI episode in children under five, of which 3.4 million were severe causing up to 199 000 deaths.[11] In addition to the health burden of viral respiratory tract infections (RTIs), the economic impact is also high if we account for health care costs (direct cost) and loss of productivity (indirect cost). A study by Fendrik et al. estimated the total economic impact of non-influenza-related viral RTIs in the United States at $40 billion annually.[12]

The advancements that have been achieved in developing antiviral drugs, some of which have already been approved, against respiratory viruses allow for targeted therapy of viral ARIs.[13-15] This possibility calls for better and faster diagnosis of the etiologic agents in ARI patients to benefit from the full potential of these drugs.[6]

Furthermore, ARIs are associated with the greatest amount of excess use of antibiotics that has led to unprecedented increase in antimicrobial drug resistance;[16,17] therefore, proper and timely diagnosis of viral infections can help reduce unnecessary antibiotic prescriptions.[5,6]

In Lebanon, studies investigating the viral etiologies of ARIs are very scarce. In this study, we determined the viral etiologies among ARI patients at a tertiary care hospital that serves an ethnically and socio-economically diverse patient population.

Materials and Methods.

Patients and samples collection. Infants and children younger than 18 years of age with symptoms of ARI disease presenting to the emergency department or the departments of pediatrics of the American University of Beirut Medical Center (AUBMC), Beirut, Lebanon were prospectively recruited between September 2009 to February 2012. An ARI was defined as an acute infection of the upper and lower respiratory airways. Recruited patients had one or more of the following symptoms: fever, cough, sore throat, rhinorrhea, headache, conjunctivitis, wheezing, dyspnea, and vomiting.

Medical history and demographic data were obtained from the patients and their medical records. A respiratory sample was collected and stored at -80°C for viral assessment. The study was approved by the Institutional Review Board (IRB) of the AUBMC, and written informed consent was obtained from all parents.

Nucleic acid extraction and viral detection. Nucleic acid was extracted from clinical specimens by using the QIAamp MinElute Virus Spin kit (Qiagen) according to the manufacturer's protocol. A 200 µl aliquot of each specimen was used for nucleic extraction. Specimens were then analyzed by the ResPlex II panel (Qiagen) using the manufacturer's protocol.

The ResPlex II panel can detect 11 viral targets: RSVA, RSVB, INFA, INFB, PIV1, PIV2, PIV3, PIV4, hMPV, CVEV (coxsackievirus and echovirus), and RhV. Briefly, 10 µl of each specimen were added to 40 µl reverse transcription-PCR (ResPlex II) master mix, including the supplied primers. Targets were detected by mixing 5 µl portions of amplification products with ResPlex II bead in hybridization buffer at 52°C for10 min. Streptavidin-phycoerythrin conjugate was added, and mixtures were incubated at 52°C for a further 5 min before the addition of stop buffer. The samples were then analyzed on a Luminex Bio-Rad BioPlex 200 System (Bio-Rad Laboratories) using Bio-Rad BioPlex Manager software. The cutoff value for each target was determined, as previously described by Lia et al.[19]

Statistical analysis. The data were checked for completeness, and responses were coded and entered into the Statistical Package for the Social Sciences (SPSS) software version 23 for Windows, which was later used for statistical analyses.[32] Descriptive statistics were presented to summarize the study variables of interest as counts and percentages for the categorical variables and as medians and Interquartile Range (IQR) for the continuous ones. The Chi-square test was used to calculate the association between two categorical variables. Pearson's chi-square analysis with Bonferroni-Holm p-value correction was used for multiple comparisons to assess infectivity enhancing correlations. Univariate and multivariate logistic regression analyses were applied to determine which factors are associated with rhinorrhea. In the regression model, rhinorrhea was used as the dependent variable. Odds ratios and their respective 95% confidence intervals were calculated. For all analysis done, a p-value of less than 0.05 was considered statistically significant.

Results.

Patient characteristics. A total of 221 specimens were collected from children presenting with symptoms of ARI between September 2009 and February 2012 (**Table 1**). The socio-demographic characteristics of the study patients are presented in Table 1. Overall, the study consisted of 130 males (58.8%) and 91 females (41.2%) with patients' median age of 1 (IQR: 0 - 5) years. Seventy-four patients (33.5%) were children under one year of age, 105 (47.5%) were between 1 to

Table 1. Demographics of the ARI patients.

Characteristics	Patients (n=221)
Median age in years (IQR boundaries)	1 (0 – 5)
Age group:	
< 1 year	74 (33.5%)
1-6 years	105 (47.5%)
6-12 years	33 (14.9%)
12-18 years	9 (4.1 %)
Gender:	
Male	130 (58.8%)
Underlying conditions:	
Asthma	34 (15.4%)
Immune-deficiency	6 (2.7%)
Allergic Rhinitis	23 (10.4%)
Cystic fibrosis	4 (1.8%)
Chemotherapy	13 (5.9%)
Prior antibiotics	95 (43%)

IQR: interquartile range.

6 years old, 33 (14.9%) were 6 to 12 years old, and 9 (4.1%) were 12 to 18 years old. Sixty-seven (30.3%) of the children had an underlying disease (asthma, immune-deficiency, allergic rhinitis, or cystic fibrosis). At the time of diagnosis 13 (5.8%) patients were receiving chemotherapy, and 95 (43%) had received an antibiotic.

Virological characterization. Samples were screened for 11 virus targets included in the ResPlexII respiratory panel. Of the 221 ARI episodes, 116 (52.5%) were confirmed to be of viral etiology being positive for at least one of the virus targets tests (**Table 2**). The majority (n=103; 88%) of viral ARI episodes were observed in children under 6-year of age (chi-square, $p<0.05$).

Figure 1 shows the frequency of each of the viruses detected in the study population. Overall 188 viruses were detected. Rhinovirus (RhV) was the most common virus detected in 81 (69.8%) patients followed

Table 2. Epidemiologic and clinical characteristics of children with viral acute respiratory infection.

		Respiratory Viruses or Infection Status						
	Any viral etiology n=116	RSV n = 18	INF n = 13	PIV n = 24	hMPV n=3	CVEV n = 45	RHV n = 81	Coinfection (≥ 2 viruses) n=49
Age:								
< 1 year	43 (37.1)	7 (38.9)	2 (15.4)	8 (33.3)	0 (0)	19 (42.2)	30 (37.0)	18 (36.7)
1-6 years	60 (51.7)	10 (55.6)	8 (61.5)	14 (58.3)	3 (100)	24 (53.3)	41 (50.6)	28 (57.1)
6-12 years	13 (11.7)	1 (5.6)	3 (23.1)	2 (8.3)	0 (0)	2 (4.4)	10 (12.3)	3 (6.1)
12-18 years	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gender:								
Female	49 (42.2)	6 (33.3)	5 (38.5)	6 (25)	1 (33.3)	20 (44.4)	35 (43.2)	20 (40.8)
Male	67 (57.8)	12 (66.7)	8 (61.5)	18 (75)	2 (66.7)	25 (55.6)	46 (56.8)	29(59.2)
Underlying conditions:								
Asthma	18 (15.5)	3 (16.7)	4 (30.8)	3 (12.5)	1 (33.3)	7 (15.6)	12 (14.8)	9 (18.4)
Immune-deficiency	2 (1.7)	0 (0)	0 (0)	1 (4.2)	0 (0)	0 (0)	1 (1.2)	0 (0)
Allergic Rhinitis	11 (9.5)	2 (11.1)	3 (23.1)	1 (4.2)	0 (0)	5 (11.1)	5 (6.2)	4 (8.2)
Cystic fibrosis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chemotherapy	7 (6)	0 (0)	1 (7.7)	1 (4.2)	0 (0)	2 (4.4)	5 (6.2)	1 (2)
Prior antibiotics	42 (36.2)	5 (27.8)	3 (23.1)	13 (54.2)	0 (0)	20 (44.4)	27 (33.3)	15 (30.6)
Symptoms:								
Fever ≥ 38ºC	99 (85.3)	16 (84.2)	12 (92.3)	19 (79.2)	3 (100)	38 (84.4)	70 (86.4)	43 (87.8)
Cough	102 (87.9)	18 (100)	13 (100)	22 (91.7)	3 (100)	39 (86.7)	72 (88.9)	46 (93.9)
Sore throat	14 (12.1)	3 (23.1)	3 (23.1)	4 (16.7)	0 (0)	4 (8.9)	7 (8.6)	6 (12.2)
Rhinorrhea	95 (81.9)	16 (88.9)	13 (100)[a]	19 (79.2)	3 (100)	41 (91.1)[b]	65 (80.2)[a]	45 (91.8)[c]
Headache	10 (8.6)	1 (5.6)	3 (23.1)	3 (12.5)	1 (33.3)	2 (4.4)	8 (9.9)	6 (12.2)
Conjunctivitis	14 (12.1)	1 (5.6)	3 (23.1)	6 (25)	0 (0)	5 (11.1)	7 (8.6)	7 (14.3)
Wheezing	43 (37.1)	10 (55.6)	4 (30.8)	9 (37.5)	1 (33.3)	21 (46.7)	29 (35.8)	20 (40.8)
Dyspnea	53 (45.7)	12 (66.7)	7 (53.8)	16 (66.7)	1(33.0)	24 (53.3)	37 (45.7)	27 (55.1)
Vomiting	49 (42.2)	8 (44.4)	6 (46.2)	12 (50.0)	0 (0)	25 (55.6)	35 (42.0)	26 (53.1)

RSV: Respiratory syncytial virus, INF: Influenza virus, PIV: Parainfluenza virus, hMPV: human Metapneumovirus, CVEV: Coxsackie/Echovirus, RHV: Rhinovirus. a : *p* value <0.05, b: *p* value <0.01, c: *p* value <0.001.

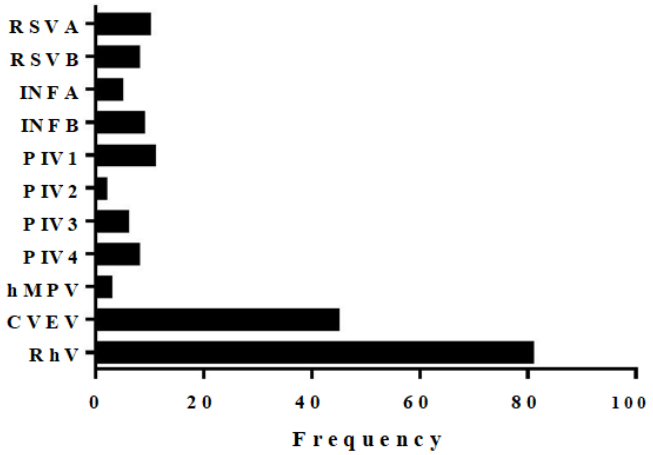

Figure 1. Distribution of viruses among 221 patients with medically attended acute respiratory infections.

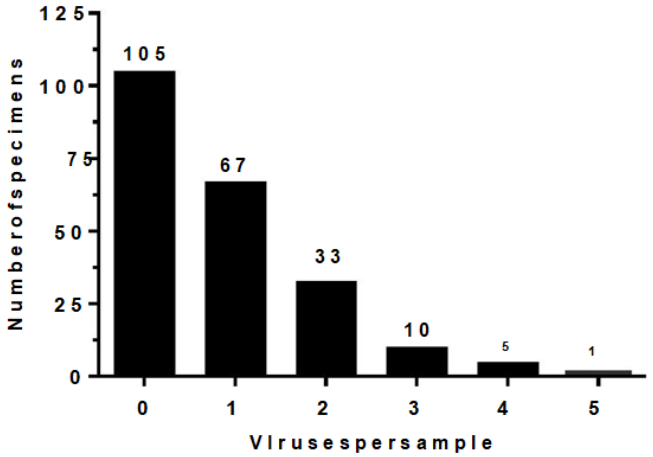

Figure 2. Prevalence of virus co-detection among the study population.

by coxsackie virus and echovirus (CVEV) which were detected as one target in the panel in 45 (38.8%) patients, parainfluenza viruses (PIV types: 1, 2, 3, 4) in 24 (20.7%) and respiratory syncytial virus (RSV types A and B) in 18 (15.5%). Coinfection with more than one virus was detected in 49 (42.9%) patients (**Figure 2**). A significant majority (n=46; 93.9%; chi-square p-value<0.05) of coinfections occurred in children under six years of age. The most frequent viral co-infections involved two viruses (n=33), 10 cases had a triple infection, 5 had four viruses detected, and one case had five viruses. Almost half cases of Rhinovirus (50.6%) were positive for at least another virus in the panel. CVEV positive cases also had a high rate of co-infection (73%). Moreover, hMPV and INFB were detected in 9 samples, and all were co-infected.

Table 3 summarizes the correlation of different viruses among our patients. Several correlations enhancing infectivity were evident in our analysis. Of note, RhV was the most frequently detected virus in co-infections and was significantly associated with RSVB, INFB, PIV3, hMPV, and CVEV.

Underlying conditions and clinical presentation. We next analyzed the correlation between each of the viral etiologies or co-infection with the underlying conditions (**Table 2**). To simplify the analysis, we treated subtypes or genotypes of a virus as a single group (e.g. RSV for RSVA and RSVB, etc.). Having an underlying condition or receiving chemotherapy or a course of antibiotics were not found to be a risk factor for having a viral etiology or co-infection (**Table 2**). Additionally, we investigated the seasonal variation of viruses. Rhinovirus infections were detected throughout the year however the peak rate occurred during the main rainy months (November, December), likely for Coxsackie/Echovirus and RSV. On the other hand,Influenza A virus infections had a peak in the fall (September, October); (**Figure 3**). In terms of clinical symptoms, fever, cough and rhinorrhea were major symptoms observed in most of the patients infected with one or more respiratory virus (**Table 2**). Chi-square analysis revealed a significant correlation between rhinorrhea and INF, CVEV, and RhV and co-infection (**Table 2**). Bivariate logistic regression was then performed to determine the risk associated with these infections. Our analysis revealed that RhV or

Table 3. Cross-tabulation of the virus frequency among ARI patients.

Virus	RSVA	RSVB	INFA	INFB	PIV1	PIV2	PIV3	PIV4	hMPV	CVEV	RhV
RSVA	10	0	0	0	0	0	0	1	0	**5***	6
RSVB		8	0	1	0	0	1	0	0	2	**6***
INFA			5	1	1	0	**1***	0	0	1	3
INFB				9	**2***	0	**5****	1	0	2	**9****
PIV1					11	0	**3****	0	0	1	3
PIV2						2	0	0	0	1	1
PIV3							6	0	0	1	**5***
PIV4								8	0	**4***	5
hMPV									3	0	**3***
CVEV										45	**28****
RhV											**81**

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

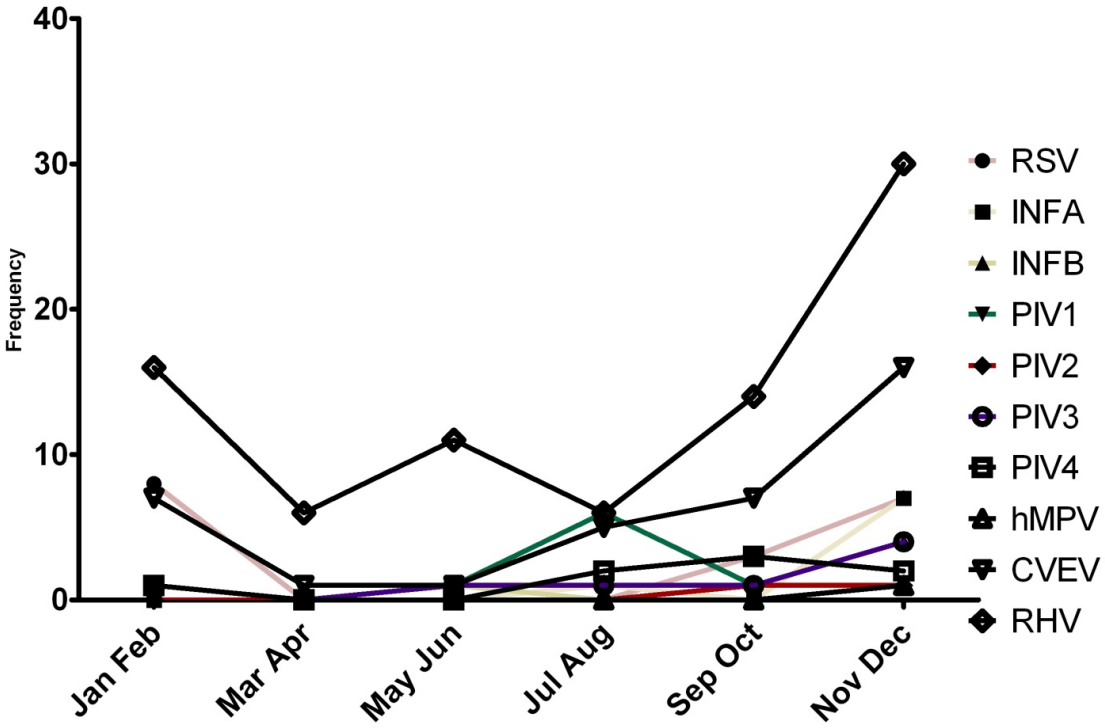

Figure 3. Seasonal distribution of viruses. The figure describes the seasonal variation of respiratory viruses in the positive pediatric samples.

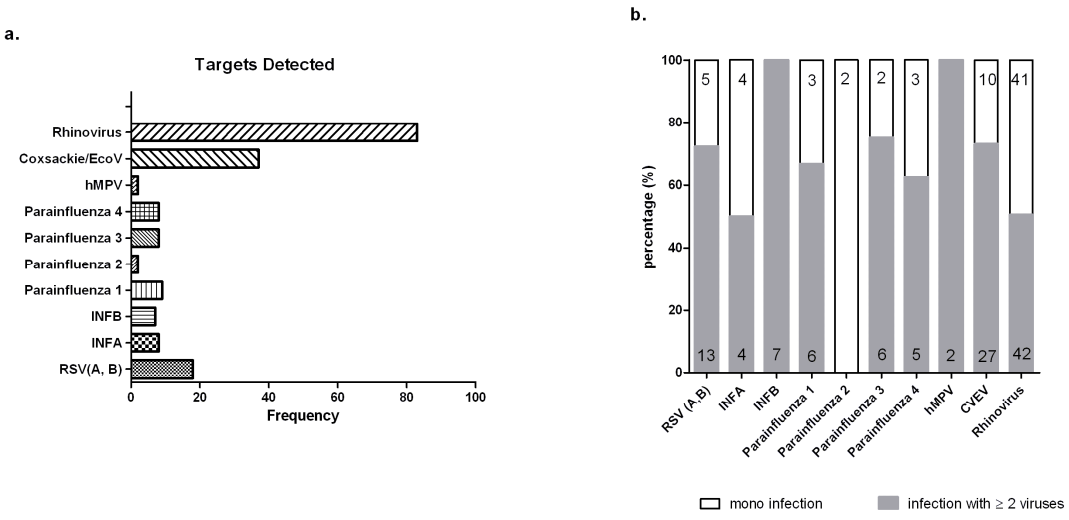

Figure 4. A. Distribution of positive signals on 10-plex panels of ResPlex II assay. **B.** Frequencies of virus detected as a single or in combination with other viruses. Numbers in bars represent the absolute numbers of infection per virus. RSV, respiratory syncytial virus; INFA, Influenza type A virus; INFB, Influenza type B virus; hMPV, human metapneumovirus; CVEV, Coxsackie/Echovirus.

CVEV infected patients or patients infected with more than one virus were more likely to have rhinorrhea (OR for RhV: 2.25; CI: 1.18 - 4.31; OR for CVEV: 5.57; CI: 1.90 – 16.28; OR for co-infection: 6.34; CI: 2.17-18.44).

A multivariate logistic regression model was used to examine the correlates of rhinorrhea in the study patients. Variables were put in the model in order of strength of their correlation with rhinorrhea as per the bivariate analysis. The effect of each variable on the model was assessed, and the variable was kept if it significantly contributed to a better fit of the model. The final model included the following variables: RhV and CVEV. The results of the multivariate model showed that CVEV was independently associated with rhinorrhea (OR: 4.73; CI: 1.59 – 14.07). CVEV infected patients were 4.73 times more likely to have rhinorrhea compared to none-CVEV patients controlling for RhV. Unlike the bivariate analysis, RhV was not significantly associated with rhinorrhea (OR:

1.78; CI: 0.91 – 3.48). RhV infected patients were 1.78 times more likely to have rhinorrhea compared to none-RhV patients controlling for CVEV; however, this was not statistically significant.

Discussion. We demonstrated that viral infections are responsible for at least half of the ARIs in children in Lebanon. Rhinovirus infection was the most common etiology of ARI consistent with other studies from Lebanon and other countries.[18,20,21] In neighboring Jordan and Egypt rhinovirus incidence was second to RSV, but the population captured in these studies was younger than that included in our study.[22,23] The overall viral ARI incidence (52.5%) in our study lower than that recently reported by Finianos et al. (70%) in Lebanon.[18] Both studies targeted children; however, Finianos et al. screened their specimens for more viral targets than those included in our analysis. In our study we did not test for HCoV, AdV, EV, and HBoV which collectively accounted for 50% of viral ARI in the study by Finianos et al.

The coinfection rate in our study (42.9%) was higher than that previously reported in Lebanon (37%), Qatar (21.4%), and Egypt (10.8%).[18,23,24] This incongruence could be because CVEV, which was frequently detected with other viruses in our study, was not screened in the previous studies from the region.[18] CVEV infections are not commonly reported in studies investigating respiratory infections. In our study, CVEV infection constituted 38.8% of all viral ARI cases and was independently associated with rhinorrhea. This incidence is much higher than that reported in other countries. A recent study in Latin America reported that CVEV was associated with 3% of the ARI cases.[25] In Central America, CVEV was even much lower (0.3%).[26] The very low prevalence of CVEV in other regions might have discouraged its testing. Given the high prevalence of CVEV in Lebanon, we recommend testing for enteroviruses, including (CVEV).

Co-infections were found to be more common younger children in Lebanon, and that is similar to a previous study done in Mexican children showing that the majority of coinfections occur in children <6 months of age.[27] Younger children are likely to be more prone to infections due to their lack or still weak immunity to respiratory viruses. The effect of coinfections on disease outcomes is not well understood.[28] Patients coinfected with pandemic H1N1 influenza and rhinovirus tended to have milder clinical severity when compared with non-rhinovirus coinfections;[29] while the patients coinfected with HMPV and RSV were prone to a higher risk of severe bronchiolitis.[30] Additionally, the prevalence and severity of obstructive airway disease were higher in patients with coinfections.[31] In our study, coinfection was associated with higher risk of rhinorrhea but not with more severe symptoms like dyspnea. In contrast, some studies showed no correlation between coinfection status and clinical severity.[32,33]

The complexity of viral coinfections and the large number of respiratory viruses involved make challenging to study the effect of coinfection on disease outcome in a clinical setting. Therefore, there is a need for developing in vitro or in vivo models to allow a better understanding of coinfections. For example, dual infection with INF was shown to suppress RSV growth in vitro.[34] The suppression of RSV by INF was suggested to be due to competition for protein synthesis and budding from the cell surface. Further studies are warranted to investigate the interactions among respiratory viruses during coinfection and their effect on the host.

Conclusions. Our study had a couple of limitations. First, we have not screened for HBoV, and HCoV which are not included in ResPlex II kit and thus the prevalence of viral ARI is expected to be higher than 52%. Another limitation was our inability to rule out bacterial etiologies which was not tested for in the current study. In conclusion, viral etiologies contribute to a large proportion of ARIs many of which involve more than one viral agent.

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