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Original Article

Viral etiologies of acute respiratory tract infections among hospitalized children — A comparison between single and multiple viral infections

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Abstract *Background:* Acute respiratory tract infections are commonly caused by viruses in children. The differences in clinical data and outcome between single and multiple viral infections in hospitalized children were analyzed.
Methods: We retrospectively reviewed the medical records of hospitalized children who had fever and a xTAG Respiratory Virus Panel (RVP) test over a 2-year period. The clinical data were analyzed and compared between single and multiple viral infections. Viral etiologies in upper and lower respiratory infections were analyzed and compared.
Results: A total of 442 patients were enrolled. Patients with positive viral detection (N = 311) had a significantly lower rate of leukocytosis (p = 0.03), less evidence of bacterial infection (p = 0.004), and shorter duration of hospitalization (p = 0.019) than those with negative viral detection. The age of patients with multiple viral infections was younger than those with single viral infection; however, there were no significant differences in duration of fever, antibiotics treatment and hospitalization between these two groups.

The most commonly identified virus was human rhinovirus. About 27% (n = 83) of patients had multiple viral infections. Overall, the highest percentage of human bocavirus infection was detected in multiple viral infections (79%). Lower respiratory tract infection (LRTI) was

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independently associated with multiple viral infections (p = 0.022), respiratory syncytial virus (RSV) infection (p = 0.001) and longer hospitalization duration (p = 0.011).
Conclusion: Multiple viral infections were associated with younger age and a higher risk of developing LRTI. However, multiple viral infections did not predict a worse disease outcome. More studies are needed to unveil the interplay between the hosts and different viruses in multiple viral infections.

Abbreviations

RVP	respiratory virus panel
LRTI	lower respiratory tract infection
RSV	respiratory syncytial virus
ARTI	acute respiratory tract infections
URTI	upper respiratory tract infection
hMPV	human metapneumovirus
HCoV	human coronavirus
HBoV	human bocavirus
ICU	intensive care unit
HAdV	human adenovirus
AOM	acute otitis media
Flu A	influenza A virus
Flu B	influenza B virus
PIV	human parainfluenza virus
EV	enterovirus
HRV	human rhinoviruses
CI	confidence interval
CRP	C-reactive protein

Introduction

Acute respiratory tract infection (ARTI) is a leading cause of fever and hospitalization in children worldwide.[1] Upper respiratory tract infection (URTI) is the most frequent human illness and the most common disease in childhood; on average, infants and preschoolers develop six to eight URTIs per year.[2] URTIs can cause non-severe but widespread epidemics that are responsible for the continuous circulation of pathogens in the community. Moreover, lower respiratory tract infection (LRTI) may also develop severe disease and is the leading infectious cause of death in children younger than five years of age.[3] For both URTI and LRTI, viral infection is the primary cause, especially in children. Clinically, the febrile presentation could lead to the use of more diagnostic tools and antibiotics. Therefore, accurate and timely identification of the responsible viral pathogen could reduce unnecessary use of antibiotics and length of hospital stay.[4]

Multiple viral infections are more commonly seen in pediatric patients than in adults.[5] Multiple viral infections were reported to be associated with increased hospital admissions, intensive care unit admissions, lengthened duration of hospitalization, and prolonged mechanical ventilation use.[6–8] However, some studies indicated that disease severity, management, and outcome were not associated with multiple viral infections.[9–12] Interestingly, a study published by Martin et al. revealed that illnesses with multiple virus detections were correlated with disease of less severity.[13] Hence, whether multiple viral infections correlate with disease severity remains unclear.

In comparison to traditional viral culture and polymerase chain reaction detection for each viral pathogen, the Luminex xTAG Respiratory Virus Panel (RVP) assay offers simultaneous testing for several common respiratory viruses with improved diagnostic sensitivity along with comparable sensitivity to individual real-time nucleic acid tests.[14] The sensitivity and specificity of RVP were reported to be 95.2% and 99.6%, respectively.[15] Furthermore, RVP can detect the presence of new viral pathogens which are not routinely cultivated or tested in clinical laboratories, such as human metapneumovirus (hMPV), human coronavirus (HCoV), and human bocavirus (HBoV).

In this study, we aim to analyze the clinical data of hospitalized children who were febrile and had a RVP test and compare the differences between patients with negative, single and multiple detections for viral pathogens. The viral etiologies contributing to single or multiple infections were compared as well as the data of patients with URTI and LRTI.

Materials and methods

Patients

We retrospectively reviewed the electronic medical records of hospitalized patients with a report of xTAG RVP from December 30, 2016, to December 31, 2018. Those enrolled patients had fever with or without respiratory symptoms on the general ward or intensive care unit (ICU) of Taipei Veterans General Hospital. RVPs were performed to investigate the possible viral etiology. Of note, three rapid antigen tests, including influenza, respiratory syncytial virus (RSV), and adenovirus (HAdV) were available on the ward. If one of the quick antigen tests came back positive, further RVP test may not be conducted. The clinical characteristics, laboratory data, comorbidities (congenital heart disease, chronic respiratory disease, chronic neurological disease, prematurity, malignancy), clinical diagnosis, duration of antibiotics use, ICU admission and duration hospitalization of the patients were recorded. Patients were divided into three age groups ($\leq$2 years old; $>$ 2 and $\leq$5 years old; $>$5 years old).

For further comparison of the differences between patients with URTI and LRTI, acute rhinitis, pharyngitis, laryngitis, tonsillitis, otitis media (AOM), acute sinusitis and croup were categorized as URTI whilst LRTI included acute bronchiolitis, bronchitis, and pneumonia. Patients with positive results of *Mycoplasma pneumoniae* serum IgM, urinary pneumococcal antigen, or bacterial culture results from blood, urine, or sputum were recorded as evidence of bacterial infection. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (No. IRB:2018-02-014AC).

Respiratory virus detection

The extraction of nucleic acids from nasopharyngeal or bronchoalveolar lavage specimens was done using QiaAmp Viral RNA/DNA Mini Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. The extracted nucleic acids were immediately frozen at −80 °C until further testing. A commercial kit, xTAG RVP Fast Assay, Version 2.0 (Luminex Molecular Diagnosis Inc. Toronto, Canada), was used for respiratory viral detection. xTAG RVP FAST v2 includes two controls (MS-2 and Lambda DNA) to ensure assay performance. This kit allows simultaneous screening for 18 common respiratory viral pathogens and subtypes in the nucleic acid samples, including influenza A virus (Flu A subtype H1, H3, and 2009 H1N1), influenza B virus (Flu B), respiratory syncytial virus (RSV), human coronaviruses (HCoVs, NL63, OC43, 229E, and HKU1), human parainfluenza viruses (PIV) type 1−4, human metapneumovirus (hMPV), enteroviruses and rhinoviruses (EV/HRV), human bocavirus (HBoV), and human adenovirus (HAdV). We further differentiated enterovirus from rhinovirus based on the symptoms, signs, and the confirmed diagnosis. The duration of antibiotics treatment and hospitalization were also recorded.

Statistical analysis

The summarized statistical results of the baseline characteristics of the patients were expressed as counts and percentages for categorical data and means with standard deviation. Continuous variables were analyzed using an independent t-test and one-way ANOVA, and categorical variables by the Chi-square test. All comparisons were two-tailed, and P-values less than 0.05 were considered statistically significant. Odds ratios and 95% confidence interval (CI) were calculated by univariate and multivariate logistic regression. All statistical analyses were performed using IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, IL).

Results

A total of 442 samples were sent for RVP tests from our pediatric ward during the study period with a positive rate

Table 1 Comparison of clinical characteristics, laboratory data and outcome in patients with negative & positive detection for viral pathogens.

	Negative detection for viral pathogens	Positive detection for viral pathogens	Univariate Analysis		Multivariable Analysis[b]	
			OR (95% CI)	p-value	aOR (95% CI)	p-value
Number (%)	131 (29.6%)	311 (70.4%)				
Gender (% male)	55.0%	61.1%	1.287 (0.85−1.94)	0.231	1.423 (0.90−2.24)	0.128
Age (year)	4.21 ± 5.28	2.68 ± 2.88		0.002*	0.891 (0.84−0.94)	<0.001*
≤2 y/o (n = 146)	53.4%	55.9%	1.107 (0.73−1.66)	0.627		
<2 ~ ≤ 5 y/o (n = 83)	20.6%	34.4%	2.020 (1.24−3.27)	0.004*		
>5 y/o (n = 19)	26.0%	9.6%	0.305 (0.17−0.52)	<0.001*		
Symptoms						
Fever duration (d)	5.17 ± 10.04	4.23 ± 3.94		0.303	1.020 (0.98−1.05)	0.270
Lab data						
WBC (/ul)	13,146 ± 8567	11,017 ± 5170		0.001*	1.000 (1.00−1.00)	0.030*
CRP (mg/dl)	3.42 ± 4.17	2.28 ± 3.87		0.016*	0.992 (0.93−1.05)	0.779
Evidence of bacterial infection[a]	29.0%	11.6%	0.320 (0.19−0.53)	<0.001*	0.420 (0.23−0.75)	0.004*
Outcome						
Antibiotic treatment duration (d)	5.52 ± 5.73	3.04 ± 4.88		<0.001*	1.009 (0.94−1.08)	0.805
ICU admission (%)	34.4%	17.4%	0.402 (0.25−0.63)	<0.001*	0.822 (0.44−1.52)	0.531
Hospitalization duration (d)	11.09 ± 13.99	5.53 ± 5.64		<0.001*	0.947 (0.90−0.99)	0.019*

[a] Positive results of *Mycoplasma pneumoniae* serum IgM, urine pneumococcal antigen, or bacterial culture from blood, urine, or sputum.

[b] Adjusted with comorbidity (congenital heart disease, chronic respiratory disease, chronic neurological disease, prematurity, malignancy).

Mean ± SD; OR = Odds ratio; aOR = adjusted Odds ratio; CI = confidence interval; d = day; WBC = white blood cell; CRP = C-reactive protein; ICU = intensive care unit.

*$p < 0.05$.

of 70%. There were 263 males (59.2%). No mortality was recorded in our study.

Comparison of clinical characteristics, laboratory data and outcome in patients with negative and positive detection for viral pathogens

As shown in Table 1, 311 patients (mean age 2.68 ± 2.88 years; range 3 days−16 years old) had positive detection for viral pathogens in comparison to 131 patients (mean age 4.21 ± 5.28 years; range 2 days−17 years old) with negative detection. Patients without viral detection were older and had longer duration of fever than those with positive detection for viral pathogens. In multivariable analysis, patients with positive viral detection had significantly lower rate of leukocytosis (p = 0.03), less evidence of bacterial infection (OR = 0.420, 95% CI 0.23−0.75, p = 0.004), and shorter duration of hospitalization (OR = 0.947, 95% CI 0.90−0.99, p = 0.019).

Comparison between single and multiple viral infections

The clinical characteristics, laboratory data and outcome of patients with single and multiple viral infections were compared (Table 2). Multiple viral infections accounted for 27% (83/311) of patients with a positive RVP report whereas 228 patients had single viral detection. To minimize the confounding effects of bacterial infection on outcome analysis, we excluded patients with AOM, acute sinusitis, and evidence of bacterial infection. As a result, the clinical data of 188 patients with single viral infection and 69 patients with multiple viral infections were compared. Using a multivariable logistic regression model with adjustment by comorbidity, patients with multiple viral infections were younger (OR = 0.812, 95% CI 0.70−0.96, p = 0.015) than those with single viral infection. Interestingly, there was no statistically significant difference in the duration of fever, antibiotics treatment and hospitalization between patients with single and multiple viral infections.

Number of identified viruses and their presence in multiple viral infections

As shown in Fig. 1, HRVs (36%, n = 113) are the most commonly identified virus among all the 311 patients, followed by RSVs (28%, n = 88), and PIVs (22%, n = 70). Notably, HBoV was detected mainly in multiple viral infections (79%, 19/24), followed by HAdV (62%, 18/29) and HRV (49%, 56/113). Among the 83 patients with multiple viral infections, the most common combination of viral infections was RSV + HRV 20.4% (17/83), followed by HRV + HBoV 8.4% (7/83) and HAdV + HRV 8.4% (7/83) (Supplemental Table 1). Moreover, dual viral pathogens were detected in 23.1% (72/311) patients with viral infections, triple in 2.2% (7/311) and quadruple in 1.2% (4/311) of patients.

Clinical characteristics, laboratory data, clinical diagnosis and outcome of patients with single viral infection

Next, we analyzed the clinical data of patients with single virus infection (Supplemental Table 2). To minimize the

Table 2 Comparison of clinical data and outcome between patients with single and multiple viral infections.

	Single viral infection	Multiple viral infections	Univariate Analysis		Multivariable Analysis[a]	
			OR (95% CI)	p-value	aOR (95% CI)	p-value
Number (%)	188 (73%)	69 (27%)				
Gender (male %)	58.6%	63.8%	1.248 (0.70−2.20)	0.446	1.233 (0.68−2.23)	0.490
Age (year)	2.76 ± 3.06	1.85 ± 1.65		0.003*	0.821 (0.70−0.96)	0.015*
≤2 y/o (n = 146)	55.8%	67.2%	1.547 (0.87−2.74)	0.134		
<2 ~ ≤ 5 y/o (n = 83)	34.6%	33.3%	0.946 (0.52−1.69)	0.853		
>5 y/o (n = 19)	10.6%	1.4%	0.124 (0.01−0.93)	0.017*		
Symptoms						
Fever duration (d)	3.84 ± 3.00	3.68 ± 2.24		0.698	1.011 (0.90−1.13)	0.852
Lab data						
WBC (/ul)	10,498 ± 4814	11,751 ± 5390		0.075	1.000 (1.00−1.00)	0.106
CRP (mg/dl)	1.77 ± 3.14	2.29 ± 3.36		0.249	1.082 (0.98−1.19)	0.114
Antibiotic treatment duration (d)	2.43 ± 3.59	2.33 ± 2.68		0.837	1.042 (0.90−1.19)	0.561
Outcome						
ICU admission (%)	19.7%	13.0%	0.612 (0.27−1.34)	0.219	0.515 (0.20−1.29)	0.157
Hospitalization duration (d)	5.12 ± 4.49	4.67 ± 2.73		0.435	0.964 (0.84−1.09)	0.575

[a] Adjusted with comorbidity (congenital heart disease, chronic respiratory disease, chronic neurological disease, prematurity, malignancy).

Mean ± SD; OR = Odds ratio; aOR = adjusted Odds ratio; CI = confidence interval; d = day; WBC = white blood cell; CRP = C-reactive protein; ICU = intensive care unit.

*p < 0.05.

We excluded patients with AOM, acute sinusitis, and evidence of bacterial infection.

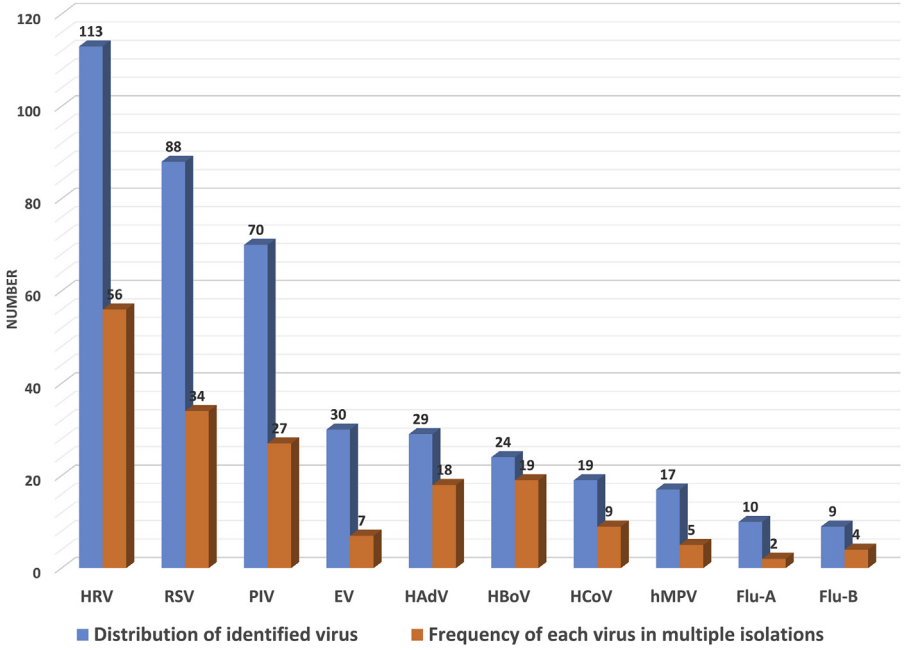

Figure 1. The number of identified viruses and their presence in multiple viral infections. Blue bars denote the number of each identified virus. Orange bars show the frequency of each virus present in multiple viral infections. PIV includes PIV1 (n = 25), PIV3 (n = 38), PIV4 (n = 6) and non-typable PIV (n = 1).

confounding effects of bacterial infection on outcome analysis, patients with AOM, acute sinusitis and evidence of bacterial infection were excluded. As a result, HAdV infection was noted more in older children (5.65 ± 3.56 years) whilst HBoV infected patients at a younger age (0.88 ± 0.09 years). Moreover, patients infected with HAdV had a longer duration of fever (5.60 ± 0.54 days) in comparison to the shorter duration of fever (2.00 ± 1.00 days) caused by HBoV infection. Notably, HAdV infection raised a higher C-reactive protein (CRP) level (3.53 ± 4.51 mg/dL). There was no single viral pathogen correlating with longer antibiotics treatment, more ICU admission or longer duration of hospitalization.

Comparison of clinical data, viral etiologies and outcome between patients with URTI and LRTI

We further compared the clinical characteristics, laboratory data, clinical diagnosis, and outcome between URTI and LTRI groups (Table 3). After patients with AOM, acute sinusitis, herpangina and evidence of bacterial infection were excluded from analysis, there were 58 and 167 patients in URTI and LRTI groups, respectively. Using multivariable logistic regression with comorbidity adjustment, we identified three factors independently associated with LRTI, including multiple viral infections (OR = 2.996, 95% CI 1.17–7.65, p = 0.022), RSV infection (OR = 6.477, 95% CI 2.17–19.27, p = 0.001), longer hospitalization duration (OR = 1.306, 95% CI 1.06–1.60, p = 0.011). By contrast, Flu-B infection (OR = 0.060, 95% CI 0.006–0.57, p = 0.015) and HCoV infection (OR = 0.255, 95% CI 0.06–0.96, p = 0.044) were independently associated with URTI. There was no statistical difference in duration of fever, CRP level, and duration of antibiotics treatment between URTI and LRTI infections.

Discussion

In the present study, we analyzed the data of 442 hospitalized children who were febrile and had the xTAG RVP test in a single medical center over a 2-year period. Patients with negative detection for viral pathogens had more evidence of bacterial infection, higher white blood cell count and longer duration of hospitalization in comparison to patients with positive detection for viral pathogens.

The age of patients with multiple viral infections was younger than those with single viral infection. Nevertheless, there was no difference in duration of fever and the clinical outcome, including duration of antibiotics treatment and hospital stay, between these two groups of patients. These findings are consistent with recent systematic reviews and meta-analyses which have concluded that there was no significant difference between children with single and multiple respiratory viral infections concerning the clinical disease severity, the length of hospital stay, admission to the intensive care unit, need for mechanical ventilation, oxygen requirements, and death.[11,16,17] By contrast, some studies showed that multiple viral infections increased the risk of ICU admission.[18]

Of note, the simultaneous detection of multiple viruses does not necessarily implicate the pathogenic effect at the time of detection, especially when molecular methods are used. It is also difficult to distinguish between active viral infection, viral shedding, and potentially non-pathogenic viral infection. For instance, HBoV and HAdV may have prolonged viral shedding.[11] The detection of two viruses

Table 3 Comparison of clinical data, viral etiologies and outcome between patients with upper and lower respiratory tract infection.

	URTI[a]	LRTI[b]	Univariate Analysis		Multivariable Analysis[c]	
			OR (95% CI)	p-value	aOR (95% CI)	p-value
Number, n	58	167				
Gender (Male: female)	55.2%	62.3%	1.341 (0.73–2.45)	0.341	1.721 (0.83–3.53)	0.140
Age (year)	2.73 ± 2.79	2.36 ± 2.53		0.357	0.987 (0.85–1.13)	0.860
≤2 y/o (%)	56.9%	58.1%	1.050 (0.57–1.92)	0.875		
>2 y/o ~ ≤ 5 y/o (%)	32.8%	35.3%	1.121 (0.59–2.11)	0.723		
>5 y/o (%)	10.3%	6.6%	0.611 (0.21–1.73)	0.351		
Pathogen						
Multiple viral infections (%)	15.5%	30.5%	2.394 (1.09–5.23)	0.026*	2.996 (1.17–7.65)	0.022*
HAdV (%)	10.3%	7.8%	0.732 (0.26–2.02)	0.546		
Flu A (%)	8.6%	1.8%	0.194 (0.04–0.83)	0.016*	0.189 (0.03–1.08)	0.061
Flu B (%)	5.2%	1.2%	0.222 (0.03–1.36)	0.077	0.060 (0.006–0.57)	0.015*
PIV (%)	32.8%	19.8%	0.505 (0.25–0.98)	0.043*	0.620 (0.27–1.41)	0.258
PIV1 (%)	15.5%	6.6%	0.384 (0.15–0.98)	0.040*		
PIV3 (%)	17.2%	10.8%	0.580 (0.25–1.34)	0.199		
PIV4 (%)	1.7%	1.8%	1.043 (0.10–10.2)	0.971		
hMPV (%)	3.4%	6.0%	1.783 (0.37–8.39)	0.458		
HCoV (%)	12.1%	4.2%	0.319 (0.10–0.95)	0.032*	0.255 (0.06–0.96)	0.044*
RSV (%)	8.6%	41.3%	7.463 (2.83–19.6)	<0.001*	6.477 (2.17–19.27)	0.001*
HRV (%)	34.5%	41.9%	1.371 (0.73–2.55)	0.319		
EV (%)	1.7%	0.0%		0.089		
HBoV (%)	5.2%	8.4%	1.678 (0.46–6.06)	0.425		
Symptoms						
Fever duration (d)	3.93 ± 3.41	3.89 ± 2.72		0.931		
Lab data						
WBC (/ul)	11,307 ± 5738	10,743 ± 4856		0.469		
CRP (mg/dl)	2.03 ± 2.87	1.97 ± 3.40		0.907	0.985 (0.87–1.10)	0.798
Outcome						
Antibiotic treatment duration (d)	1.52 ± 2.28	2.53 ± 3.56		0.044*		
ICU admission (%)	5.2%	18.6%	4.179 (1.22–14.2)	0.014*	3.308 (0.82–13.28)	0.092
Hospitalization duration (d)	3.53 ± 1.76	5.08 ± 4.36		0.009*	1.306 (1.06–1.60)	0.011*

[a] We excluded patients with AOM, acute sinusitis, herpangina and evidence of bacterial infection.
[b] We excluded patients with evidence of bacterial infection.
[c] Adjusted with comorbidity (congenital heart disease, chronic respiratory disease, chronic neurological disease, prematurity, malignancy).
Mean ± Standard deviation; OR = Odds ratio; aOR = adjusted Odds ratio; CI = confidence interval; d = day; URTI = upper respiratory tract infection; LRTI = lower respiratory tract infection; WBC = white blood cell; CRP = C-reactive protein; ICU = intensive care unit.
*$p < 0.05$.

may represent an episode of acute infection in the presence of other viral persistence from a recent infection.[19] Moreover, the mechanisms of disease virulence and viral–viral interaction in multiple infections are not clearly understood. They may result from direct interactions of viral genes or indirect interactions resulting from alterations in the host-environment or immunological interactions.[20] Different pathogenic mechanisms may be triggered by different viruses, which may potentiate or inhibit the effects on each other. For instance, RSV or influenza coinfection with other viruses may associate with a worse outcome.[7,21] Furthermore, some studies indicated a high viral load is related to higher disease severity in respiratory infections[22]; however, conflicting results have been reported.[23] Therefore, further studies are needed to determine whether multiple viral infections contribute to disease severity.

Of all the respiratory viruses analyzed, HRV, RSV, and PIV were the most commonly identified viruses in descending order. Previous studies have shown slightly different rankings of common respiratory pathogens; nevertheless, HRV has consistently been the most common cause in all age groups.[24] Similar to results of previous studies, RSV and PIV are the most common cause of lower respiratory tract infections in infants and young children.[25,26] HAdV is accountable for around 10 percent of ARTI in children in the previous study[5] and it was 9% in our study. This was underestimated because patients with a positive quick adenovirus antigen test without proceeding to have xTAG RVP testing were not included in our analysis. Six percent (19/311) of children were reported positive for HCoV, of which 2.5% were positive for OC43 (8/311), 2.2% for NL63 (7/311), 0.6% for 229E (2/311), and 0.6% for HKU1 (2/311). These are similar to previous results.[27]

In this study, 27% of children had multiple viral infections. The result is similar to the previous studies that detection of multiple viruses simultaneously in pediatric patients ranges from 10 to 30%.[28] Similar to previous findings, the three most commonly detected viruses in multiple viral infections were HRV, RSV, and PIV in this study.[10,29] Furthermore, higher percentages of HBoV (79%), HAdV (62%), and HRV (49%) infection were present in children with multiple viral infections. Previous studies showed that around 33%—75% of HBoV was detected in children who had co-infection with other viruses.[30,31] According to the previous study by Lee et al., multiple respiratory viral infections with HAdV were reported in 30.5% of children.[32] HRV was the most common co-infecting virus which occurring in 69.6% of multiple viral infections.[33] In addition, the most common combinations of multiple viral infections were HRV + RSV and HRV + HAdV and triple/quadruple viral infections accounted for a certain proportion similar to the results of previous studies.[11,33—35]

HBoV infections occurred mostly in children less than 2 years of age with a mean age of 0.88 ± 0.09 years. The previous study indicated that HBoV circulates in the community and is acquired early in life.[36] The clinical picture of adenovirus infection was characterized by high grade and prolonged fever. Laboratory findings of HAdV infections include elevated CRP, leukocytosis, and neutrophilia.[37] In our study, HAdV had significantly longer fever duration (5.60 ± 0.54 days) and elevated CRP level (3.53 ± 4.51 mg/dl).

Flu-B infection and HCoV infection were independently associated with URTI whereas multiple viral infections, RSV infection, and longer hospitalization duration were independently associated with LRTI. To minimize the confounding effects of concurrent bacterial infection, we excluded patients with AOM, acute sinusitis, and evidence of bacterial infection. Those patients with a positive influenza antigen test were not enrolled for analysis so the case number was small. Previous studies have shown four subtypes of HCoV (OC43, 229E, NL63, HKU1) lead to milder URTI.[38] On the other hand, RSV has been reported as the primary pathogen causing LRTI and hospitalization in young children.[39] In our study, 90% of patients with positive RSV detection had acute bronchiolitis or pneumonia.

There were several limitations to this study. Firstly, those who had a positive quick antigen test result without RVP testing were not included. That could explain why the numbers of cases with adenovirus, RSV and influenza infections were smaller in comparison to previous studies.[5,29] Secondly, the RVP was not a timely test as it might take days to obtain the result. The delay might have contributed to the prolonged course of antibiotics use or lengthened hospital course due to fever. Thirdly, patients with bacterial co-infection but without evidence potentially have a confounding influence on the outcome analysis.

In conclusion, the RVP has unveiled many viral pathogens that have not been routinely detected or cultured in clinical settings. In comparison to patients with negative detection for viral pathogens, those with positive detection for viral pathogens had significantly lower rates of leukocytosis, evidence of bacterial infection, and shorter duration of hospitalization. Multiple viral infections were more common in younger children and did not predict a worse outcome such as duration of fever and hospitalization. The most common identified virus remained HRV whilst HBoV was present in a high percentage in multiple viral infections. Moreover, LRTI was independently associated with multiple viral infections, RSV infection and longer hospitalization duration. Further studies on the pathogenesis, disease severity and outcome of different viral combinations are needed to clarify the interplay of host and viruses in multiple viral infections.

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Contributors' statement

C.-Y. Yen, Y.-J. Chan, K.-G. Wu and M.-C. Hung conceived the idea of this study and designed the study. C.-Y. Yen and W.-T. Wu conducted data acquisition and performed statistical analysis. C.-Y. Yen, C.-Y. Chang, Y.-C. Wong and C.-C. Lai drafted the initial manuscript. C.-Y. Yen K.-G. Wu and M.-C. Hung critically revised the manuscript. All authors approved the final version of the manuscript as submitted and agree to be accountable for all aspects of the work.

Declaration of Competing Interest

All authors have no conflicts of financial and non-financial interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2019.08.013.