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Original Article

Molecular epidemiology and clinical features of rhinovirus infections among hospitalized patients in a medical center in Taiwan

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Abstract *Background:* Human rhinovirus (HRV) can cause severe illnesses in hospitalized patients. However, there are no studies regarding the prevalence of HRV infection, particularly the recently identified HRV-C, in hospitalized patients reported from Taiwan.
Methods: Respiratory specimens collected from 487 hospitalized patients in designated wards between 2013 and 2014 in a medical center in northern Taiwan were retrospectively detected for HRV. Positive specimens were further determined for genotyping. Medical charts of the HRV-positive patients were reviewed retrospectively.
Results: Totally, 76 patients (15.6%) were HRV positive, of which 60 were pediatric patients. HRV-A was identified in 41 (54%) patients, HRV-B in 6 patients (7.9%) and HRV-C in 29 patients (38%). A total of 47 different genotypes were identified. HRV infections were predominant during fall and winter seasons. 21.1% were affected by HRV alone and 78.9% were found to be co-infected with other microorganisms. The detection rate of HRV in children (18.6%) was significantly higher than in adults (9.6%). Compared with pediatric patients, adult patients were

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significantly associated with underlying disease, *Pneumocystis jirovesii* pneumonia co-infection, a diagnosis of pneumonia, fatal outcome, hospital acquisition of HRV, antibiotics administration and requiring intensive care, while pediatric patients were significantly associated with viral co-infection.

Conclusions: HRV was a common cause of respiratory tract infection in Taiwan, particularly in pediatric patients. Eighty percent of HRV-infected inpatients had other microorganisms co-infection. Adult patients were more likely to be associated with a severe respiratory disease entity.

Introduction

Human rhinoviruses (HRVs) were first identified in the 1950s. HRVs are members of the Enterovirus genus, family *Picornaviridae*, and are further characterized as three species (HRV-A, -B, and -C).[1,2] It is a small single-stranded RNA virus, about the size of a ribosome.[3,4] The capsid has icosahedral symmetry and contains 60 copies each of the four rhino-viral polypeptides (VP1∼VP4)[1].

Approximately 100 serotypes in two distinct groups (HRV-A and HRV-B) were identified by 1990s. The development of modern molecular techniques for the identification of HRV led to the identification and designation of a novel species, HRV-C in 2009. HRVs are transmitted from person to person via contact or aerosol. HRV are the most frequent cause of respiratory tract infections in humans.[5–7] Although more often associated with mild upper respiratory tract infections, HRVs were recognized as a major cause of lower respiratory tract infections, including pneumonia, bronchiolitis and asthma.[8–10]

Similar to other viruses, the epidemiology as well as molecular epidemiology of HRV varied geographically in different countries and regions.[11,12] However, there was no report regarding the prevalence and epidemiology of HRVs, particularly the recently identified HRV-C, in hospitalized patients from Taiwan. We conducted this study to determine the prevalence and epidemiologic features of HRVs infections, and to assess the associations between HRV species and demographic, and clinical features in hospitalized patients in Taiwan.

Methods

Population

From January 2013 to December 2014, pediatric patients age less than 18 years and adults with acute respiratory tract infections and a specimen from respiratory tract (including throat swab, sputum, bronchoalveolar lavage or nasopharyngeal swab) sent for virus isolation and identification in Chang Gung Memorial Hospital were enrolled in this study. The microorganism examinations such as blood culture, sputum culture, gram's stain, virus isolation were performed in adult patients; virus isolation and blood culture were sent in pediatric patients. In addition, bacterial culture and virus isolation from bronchoalveolar lavage were sent in patients with ventilator support. All patients were selected from designated divisions, which included pediatric intensive care unit, division of pediatric infectious diseases, division of pediatric pulmonology, division of pediatric rheumatology and pediatric emergent department for pediatric patients and division of infectious diseases, division of pulmonology and medical intensive care units for adult patients. From a single patient only one specimen was selected. Medical charts of the HRV-positive patients were reviewed retrospectively. The demographics, underlying disease, the duration of hospitalization, clinical manifestations, laboratory data, final diagnosis, clinical outcome and complications were collected.

Definition

Pneumonia was defined as patients with cough or difficult breathing, tachypnea and/or chest in-drawing, and a dense opacity on chest radiographs. Bronchopneumonia was defined as an acute inflammation of the lungs, characterized by cough, fever, high respiratory rates and bronchial breathing without opacity on chest radiographs. Co-infection was defined as other microorganisms infected within 48 h of Rhinovirus Infection. Bacterial co-infection was defined as specimens from blood, sputum, bronchoalveolar lavage or other sterile sites yielding clinically significant bacteria in patients with positive results for HRV. The sputum culture was only evaluated when Gram's stain revealed numerous leukocytes ($>$25 in a 100 $\times$ microscopic field) and few epithelial cells ($<$10 in a 100 $\times$ microscopic field). An organism presenting heavy growth ($\geq 10^7$ colony forming units [CFU]/mL) of a predominant bacterium on sputum culture was considered to be a pathogen. When Gram staining revealed a bacterium compatible with the culture results, moderate growth (10^5 or 10^6 CFU/mL) in the sputum culture was also considered to be evidence of a presumptive pathogen.

RT-PCR and sequencing

Total RNAs were extracted using LabTurbo Viral DNA/RNA Extraction Kit (TaiGen Biotechnology Inc., Taiwan) according to the manufacturer's specifications. cDNA are synthesized

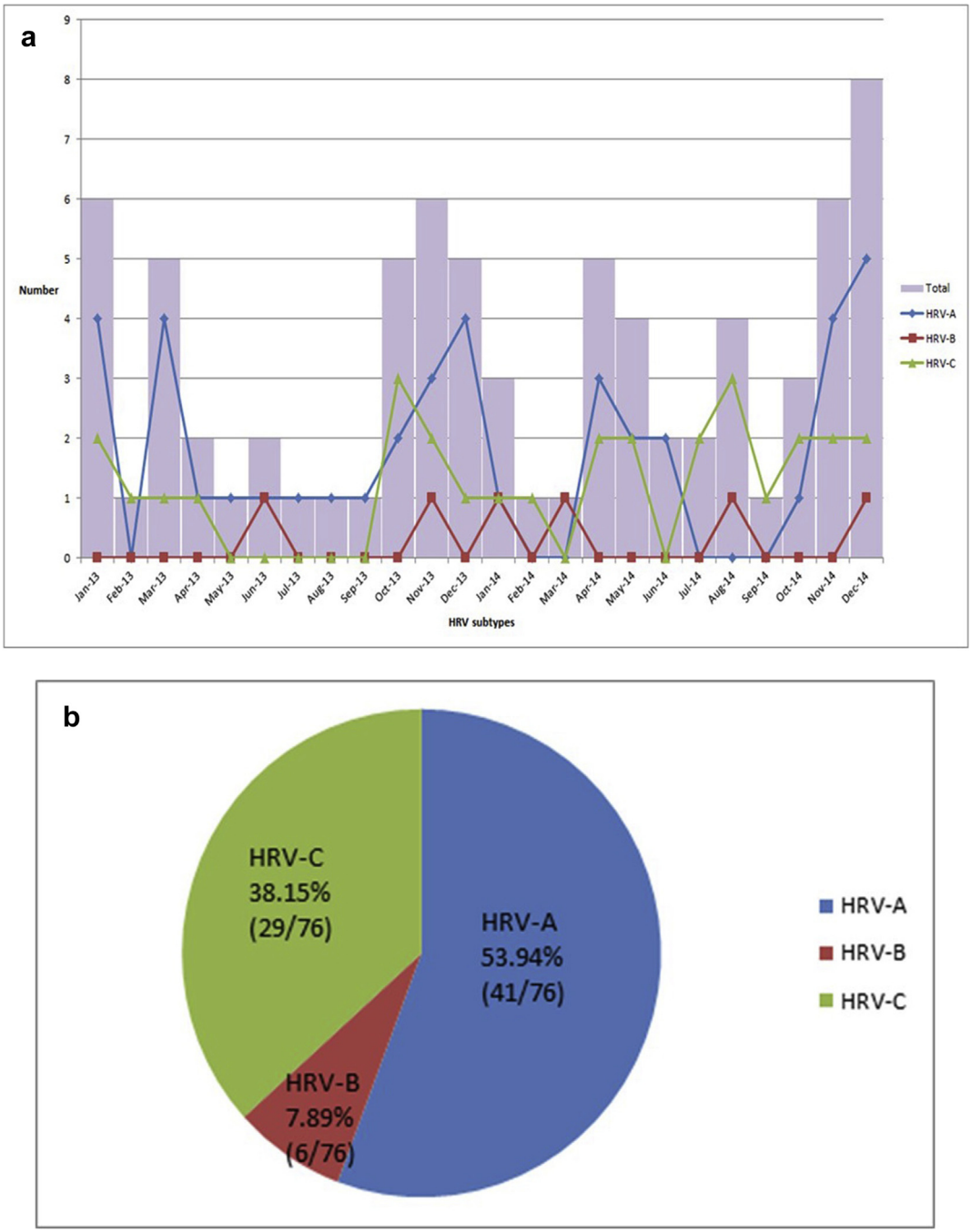

Figure 1. a Monthly distribution of human rhinovirus-positive case number during 2013 and 2014 in a medical center in Taiwan. b The percentage of different human rhinovirus species.

from total RNA using the SuperScript III reverse-transcription system (Invitrogen, USA). The VP4/VP2 sequences of the HRV strains will be amplified by semi-nested PCR with the HRV forward (F484-5′CGGCCCCTGAATGYGGCTAA3′ and F587sn-5′CTACTTTGGGTGTCCGTGTTTC3′) and reverse primers (R1126-5′ATCHGGHARYTTCCAMCACCA3′) as previously described.[13] The HRV species for each strain is identified by direct sequencing of VP4/VP2 PCR products. The PCR products are purified from agarose gel using the gel extraction kit (QIAGEN, USA) according to the manufacturer's specifications. The purified DNA will be served as templates for chain termination reaction with the ABI 3730 XL DNA Analyzer (Applied Biosystem Inc., Foster City, CA). All the amplification products were sequenced bidirectionally to confirm amplification specificity and virus typing by phylogenetic analysis.[14]

Virus isolation and identification

Clinical respiratory tract specimens were inoculated into MRC-5, RD, and MDCK cells. Cultures were maintained in minimal essential media containing

antibiotics and incubated at 35 °C. The inoculated cell cultures were maintained and observed for the presence of cytopathic effects (CPE) for at least 4 weeks. Respiratory viruses, including human adenovirus (HAdV), human metapneumoniae virus (hMPV), human parainfluenza (HPIV) type 1, 2 and 3, influenza A and B viruses (IAV and IBV), and respiratory syncytial virus (RSV) in CPE-positive cases were further identified by immunofluorescence assay with D3 Ultra DFA respiratory virus screening and identification kit (Diagnostic Hybrids, Inc., Athens, OH, USA.

Ethic statement

This study was conducted with the approval of the Institutional Review Board of Chang Gung Memorial Hospital. (CGMH; No. 100-2518B; IRB:201600874B0).

Statistical analyses

Demographic, clinical, and laboratory features (categorical variables) associated with viral co-infection were examined using Chi-squared (x2) or Fisher's exact tests. Continuous variables were analyzed using variance (ANOVA) models and presented as means with SD or medians with IQR. Variables that were not normally distributed were logarithm-transformed and presented as means. The variables with significant difference between adult patients and pediatric patients by univariate analysis were selected for further multiple regression analysis. In addition, some potential risk factors for ICU admission were investigated using multiple regression analysis. Statistical analyses were performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL) and a P-value <0.05 was considered statistically significant.

Results

Molecular epidemiology of HRV infection in hospitalized patients

A total of 487 specimens from hospitalized patients, including 321 children and 166 adults, with respiratory tract infections were selected and retrospectively screened for HRVs. The patients aged between 3 months and 82 years with a median of 3.85 years. 76 patients (15.6%), including 60 children and 16 adults, were HRV positive. The monthly HRV-positive number is shown in Fig. 1a. Generally, HRV was detected in each month with more case number between October and January. Of the 76 HRV isolates, 41 (53.9%) were HRV-A, 6 (7.9%) were HRV-B and 29 (38.2%) were HRV-C (Fig. 1b). HRV-A, -B, and -C strains shared more than 92% of nucleotide sequence similarities with their reference strains. A total of 47 different genotypes were identified from these HRV strains, as shown in Table 1.

Table 1 Distribution of HRV species and genotypes in 76 HRV-infected inpatients in Chang Gung Memorial Hospital during 2013 and 2014.

HRV specie	Genotypes
A	A07, A12, A15, A16, A20, A21, A22,A24, A28, A29, A31, A32, A34, A38, A44, A49, A55, A56, A57, A58, A60, A62, A66, A71, A73, A78, A89, A100, A101
B	B04, B06,B14, B91
C	C01,C02, C06, C07,C09, C11, C16, C17, C22, C23, C26, C31, C32, C42

Demographic data and clinical characteristics of 76 hospitalized patients infected with different HRV genotypes

The detection rate of HRV infection in children (60/321, 18.6%) was significantly higher than that in adults (16/160, 9.6%) (Table 2). Among younger children age less than 6 years, the detection rate of HRV-A and HRV-C was similar, but was higher than that for HRV-B (only 2%). The detection rate of HRV-C in these younger children was significantly higher than that in adults ($p < 0.05$) (Table 2).

The clinical and epidemiological characteristics of the HRV-positive patients are shown in Table 2. A total of 45 (59.2%) patients were male. Thirty patients (39.5%) had an underlying condition, which included chromosome anomaly, metabolic disease, neurologic disorder, congenital heart disease, hematologic disorder, congenital or acquired immunodeficiency, and oncologic disorder. A total of 15 patients (19.7%) had bacterial co-infection, while antibiotics were administered in 63 patients (82.9%). Among the 76 HRV-positive patients, 21.1% were affected by HRV alone and 78.9% were found to be co-infected with other viruses, fungi or bacteria. The most common co-infected virus was adenovirus (n = 18, 23.7%), followed by parainfluenza virus (n = 9, 11.8%) and herpes simplex virus-1 (n = 5, 6.6%) (Table 3). The demographics and clinical characteristics of patients infected by HRV with or without other viral infection are provided in supplementary Table 1. No significant differences was noted between the two groups in terms of demographics and clinical characteristics.

In HRV-positive group, 59 (77.6%) were community-acquired infection. The mortality rate was 11.2% (n = 9). Loss of appetite (n = 63, 82.9%), decreased activity (n = 59, 77.6%), fever (n = 56, 73.7%), cough (n = 56, 73.7%) and rhinorrhea (n = 46, 60.5%) were the most common symptoms (Table 4). Wheezing was more commonly seen in HRV-C group but did not reach statistically significant difference between species. Upper respiratory traction infections (URTI), which included acute tonsillitis, acute pharyngitis, acute sinusitis and acute otitis media, were diagnosed in 31 (40.8%) patients, while 45 (59.2%) were diagnosed as lower respiratory traction infections (LRTI) which included bronchiolitis, bronchopneumonia and pneumonia. Besides, much more patients were diagnosed as pneumonia in HRV-A group and as bronchiolitis in HRV-C group. The rate of ICU admission in patient with HRV-A infection was significantly higher than in patients with HRV-B or HRV-C infection ($p < 0.05$).

Table 2 Comparison of Clinical and Demographic Characteristics of HRV-Positive Patients stratified by HRV species.

Characteristics	HRV-A (n = 41) No. (%)	HRV-B (n = 6) No. (%)	HRV-C (n = 29) No. (%)	Total (n = 76) No. (%)	*p*-value
Age					
>18 years old	10 (6.0)	2 (1.2)	4 (2.4)	16 (9.6)	
≤18 years old	31 (9.6)	4 (1.2)	25 (7.8)	60 (18.6)	0.0016*
< 6 year old	23 (9.9)	4 (1.7)	22 (9.4)	49 (21)	0.0042*
6—11 year old	4 (7.1)	0 (0.0)	3 (5.3)	7 (12.4)	0.28
12—18 year old	4 (12.1)	0 (0.0)	0 (0.0)	4 (12.1)	—
Gender (male)	20 (48.7)	5 (71.4)	20 (71.4)	45 (59.2)	0.109
Underlying diseases	19 (46.3)	1 (14.2)	10 (35.7)	30 (39.5)	0.298
community-acquired infection	30 (73.1)	4 (66.7)	25 (86.2)	59 (77.6)	0.348
Nosocomial infection	11 (26.9)	2 (28.6)	4 (14.3)	17 (22.4)	—
Co-infection with any pathogen	30 (82.9)	5 (83.3)	19 (72.4)	54 (78.9)	0.68
Bacteria alone	6 (14.6)	0 (0)	7 (24.1)	13 (17.1)	—
Virus alone	22 (53.7)	4 (66.7)	13 (44.8)	37 (51.3)	—
Fungus alone	3 (7.3)	1 (16.7)	1 (3.4)	5 (6.6)	
Mixed virus and bacteria	2 (4.9)	0 (0)	0 (0)	2 (2.6)	—
Mixed virus and fungus	1 (2.4)	0 (0)	0 (0)	1 (1.3)	
Negative	7 (17.1)	1 (16.7)	8 (27.6)	16 (21.1)	—
Clinical manifestation					
Fever	31 (75.6)	4 (66.7)	21 (72.4)	56 (73.7)	0.88
Rhinorrhea	22 (53.7)	5 (83.5)	19 (65.5)	46 (60.5)	0.298
Sore throat	8 (19.5)	1 (14.2)	2 (7.1)	11 (14.5)	0.331
Nasal congestion	7 (17.0)	0 (0.0)	5 (17.9)	12 (15.8)	0.543
Cough	32 (78.0)	4 (66.7)	20 (69.0)	56 (73.7)	0.641
Wheezing	3 (7.3)	0 (0.0)	5 (17.9)	8 (10.5)	0.28
Dyspnea	20 (48.8)	1 (14.2)	15 (53.5)	36 (47.3)	0.283
Skin rash	2 (4.9)	0 (0.0)	1 (3.6)	3 (4.0)	0.836
Diarrhea	3 (7.3)	1 (14.2)	2 (7.1)	6 (7.9)	0.707
Seizure	4 (9.8)	1 (14.2)	2 (7.1)	7 (9.2)	0.741
Headache	3 (7.3)	1 (14.2)	1 (3.6)	5 (6.6)	0.474
Loss of activity	31 (75.6)	4 (66.7)	24 (82.8)	59 (77.6)	0.622
Loss of appetite	35 (85.3)	4 (66.7)	24 (82.8)	63 (82.9)	0.524
Diagnosis					0.817
URTI	15 (36.6)	3 (50.0)	13 (44.8)	31 (40.8)	—
LRTI	26 (63.4)	3 (50.0)	16 (55.2)	45 (59.2)	—
Bronchiolitis	3 (7.3)	0 (0.0)	4 (13.8)	7 (9.2)	—
Bronchopneumonia	7 (17.1)	1 (16.7)	5 (17.3)	13 (17.1)	—
Pneumonia	16 (39.0)	2 (33.3)	7 (24.1)	25 (32.5)	—
Day of fever					0.473
< 7 days	26 (63.4)	5 (83.3)	14 (48.3)	45 (59.2)	—
7—14 days	12 (29.3)	1 (16.7)	11 (37.9)	24 (31.6)	—
>14 days	3 (7.3)	0 (0.0)	4 (13.8)	7 (9.2)	—
Day of hospitalization					0.510
<7 days	6 (14.6)	2 (33.3)	9 (31.0)	17 (22.4)	—
7—14 days	16 (39.0)	2 (33.3)	8 (27.6)	26 (34.2)	—
>14 days	19 (46.3)	2 (33.3)	12 (41.4)	33 (43.4)	—
ICU admission	26 (63.4)	3 (50.0)	9 (31.0)	38 (50)	0.028*
Antibiotics administration	35 (85.4)	6 (100)	22 (75.9)	63 (82.9)	0.297
Mortality	6 (14.6)	0 (0.0)	3 (10.7)	9 (11.2)	0.556

Abbreviations: URTI = upper respiratory tract infection; LRTI = lower respiratory tract infection; ICU = intensive care unit.
*Statistical significance: $p < 0.05$.

Comparison between pediatric patients and adult patients with HRV infection

The demographic and clinical characteristics of HRV infection between adult patients and pediatric patients are summarized in Table 4 and Supplement Table 2. Adult patients were more likely to have underlying disease, *Pneumocystis jirovecii* co-infection, fatal outcome, hospital acquisition, antibiotics administration and ICU admission, whereas pediatric patients were more likely to have viral co-

Table 3 Co-infections of 76 HRV-infected patients with other microorganisms.

Microorganism	HRV-A (n = 41) No. (%)	HRV-B (n = 6) No. (%)	HRV-C (n = 29) No. (%)	Total (n = 76) No. (%)
Viruses	22 (53.7)	4 (66.7)	13 (44.8)	39 (51.3)
Adenovirus	9 (22.0)	2 (33.3)	7 (24.1)	18 (23.7)
Parainfluenza viruses	6 (14.6)	1 (16.7)	2 (6.9)	9 (11.8)
Herpes simplex virus-1 (HSV-1)	4 (10.0)	0	1 (3.4)	5 (6.6)
Respiratory syncytial virus (RSV)	2 (4.9)	0	1 (3.4)	3 (3.9)
Influenza A virus	1 (2.4)	0	0	1 (1.3)
Influenza B virus	0	0	1 (3.4)	1 (1.3)
Parainfluenza viruses & influenza A	0	1 (16.7)	0	1 (1.3)
HSV-1 & RSV	0	0	1 (3.4)	1 (1.3)
Bacteria	8	0	7	
Pseudomonas aeruginosa	3	0	2	5 (6.6)
Staphylococcus aureus	1	0	2	3 (3.9)
Acinetobacter baumannii	1	0	2	3 (3.9)
Streptococcus pneumoniae	1	0	0	1 (1.3)
Escherichia coli	0	0	1	1 (1.3)
Enterococcus faecium	1	0	0	1 (1.3)
Mycoplasma pneumoniae	1	0	0	1 (1.3)
Fungi				
Pneumocystis jiroveci	4	1	1	6 (7.9)

infection. For clinical manifestation, fever and nasal congestion were more frequently observed in pediatric group than in adult group (all $p < 0.05$), while adult patients were more frequently diagnosed as lower respiratory tract infection than pediatric patients. Six of the variables with significant difference between adult patients and pediatric patients by univariate analysis, including viral co-infection, *Pneumocystic jirovesii* co-infection, underlying diseases, nosocomial infection, antibiotic administration and ICU admission, were selected for further multiple regression analysis and the results are shown in supplement Table 3, revealing that pediatric patients were more likely to have viral co-infection. The risk factors for ICU admission by multiple regression analysis are shown in supplement Table 4. Underlying disease was positively correlated with ICU admission while viral co-infection was negatively correlated.

Discussions

To our knowledge, this is the first study regarding the epidemiological, virological and clinical characteristics of HRV infections in hospitalized patients with respiratory tract disease in Taiwan. To date, at least 77 different serotypes of HRV-A, 25 different serotypes of HRV-B and 51 different serotypes of HRV-C had been identified.[15] In this study, 47 serotypes were identified in 76 patients. HRVs are a highly diverse group of respiratory viruses.[16]

In this study, more than half of the HRV strains belonged to HRV-A, and nearly 40% of the typed HRV samples belonged to HRV-C, while less than 10% were HRV-B. The findings that HRV-A and HRV-C were the predominant species in respiratory tract disease, and HRV-B was observed sporadically were consistent with previous reports.[17–20] Approximately half of the patients with HRV infections were co-infected with other viruses, and the most common co-infectious virus was adenovirus in this study. Co-infection occurred evenly between HRV-A and HRV-C-infected patients.

Lau et al. in a review article[21] indicated that the seasonality of HRV circulation varied geographically in different countries or regions. HRV usually peaked in fall or winter in most temperate or subtropical countries.[18,20,22] Taiwan is located in subtropical zone. In this study, HRV were seen all year round with a peak in the late autumn and early winter during 2013 and 2014. Previous studies also reported that the prevalence of HRV infection differed with age.[12,23,24] HRV-C infection was more commonly seen in children and the majority of adults patients were infected with HRV-A. In this study, the positive rate of HRV as well as HRV-C infection in adult patients was significantly lower than those in children. HRV-C infection was significantly associated with younger children <6 years of age than HRV-A and HRV-B.

Previous reports indicated that the clinical manifestations varied between HRV-C and HRV-A[19,25] with HRV-C being dominant in patients with acute wheezing illness or a diagnosis of bronchiolitis while patients with HRV-A infection being increased proportions of pneumonia.[26] In the present study, no significant difference of clinical characteristics was found between HRV-C and HRV-A infection. Though wheezing was more common in HRV-C group, the difference did not reach significant difference compared to the patients with HRV-A infection. Meanwhile, HRV-A-infected patients were likely to be diagnosed as pneumonia and require intensive care while HRV-C-infected patients as bronchiolitis. ($p < 0.05$).

In this study, compared with pediatric patients, adult patients were significantly more likely to have bacterial and *P. jirovecii* co-infection, underlying illness, nosocomial infection, ICU admission, and a diagnosis of pneumonia by univariate analysis. However, by multiple regression analysis, the only significant finding was that pediatric patients were more likely to have viral co-infection. Further analysis showed that viral co-infection was negatively correlated with ICU admission, but underlying disease was positively correlated with ICU admission. In these adult patients, HRV might be a trigger agent (preceding infection), precipitating agent or even a bystander (biomarker) but could not be defined clearly. Previous studies also found that the hospitalized patients had a higher percentage of having an underlying illness than the mild or asymptomatic HRV infected patients.[20,27] In contrast, previous reports indicated that multiple viral infections are associated with a more severe

Table 4 Comparison of Demographic and Clinical Characteristics between HRV-infected Adult patients and Pediatric patients.

Characteristics	Adults (N = 16)	Pediatrics (N = 60)	*p*-value
Gender, male (%)	10 (62.5)	35 (58.3)	0.49
Bacterial co-infection	6 (37.5)	9 (15)	0.054
Pneumocystis jirovesii co-infection	5 (31.5)	1 (1.7)	0.001
Viral co-infection	1 (6.3)	38 (63.3)	<0.001
Underlying disease	13 (81.3)	17 (28.3)	<0.001
Mortality	6 (37.5)	3 (5)	0.002
Nosocomial infection	10 (62.5)	7 (11.7)	<0.001
Antibiotics administration	16 (100)	47 (78.3)	0.034
Day of antibiotics, (range)	40.2 (17–111)	10.9 (0–150)	0.152
ICU admission	14 (87.5)	24 (40)	0.001
Day of ICU, (range)	22.69 (18–92)	8.43 (0–227)	0.671
Stay of ICU			<0.001
< 7 days, n (%)	3 (18.8)	47 (78.3)	
7–14 days, n (%)	3 (18.8)	5 (8.3)	
>14 days, n (%)	10 (62.5)	8 (13.3)	
Day of hospitalization, (range)	59 (34–113)	22 (4–273)	0.981
Stay of hospitalization			<0.001
< 7 days, n (%)	0 (0)	17 (28.3)	
7–14 days, n (%)	0 (0)	26 (43.3)	
>14 days, n (%)	16 (100)	17 (28.3)	
Clinical manifestation			
Fever, n (%)	9 (56.3)	47 (78.3)	0.075
Rhinorrhea, n (%)	7 (43.8)	39 (65)	0.10
Cough n (%)	12 (75)	44 (73.3)	0.58
Nasal congestion, n (%)	0 (0)	12 (20)	0.045
Sore throat, n (%)	4 (25)	7 (11.7)	0.17
Wheezing, n (%)	0 (0)	8 (13.3)	0.136
Dyspnea, n (%)	13 (38.3)	23 (81.3)	0.002
Skin rash, n (%)	0 (0)	3 (5)	0.487
Diarrhea, n (%)	0 (0)	6 (10)	0.229
Seizure, n (%)	0 (0)	7 (11.7)	0.177
Headache, n (%)	0 (0)	5 (8.3)	0.296
Loss of activity, n (%)	10 (62.5)	49 (81.7)	0.10
Loss of appetite, n (%)	11 (68.8)	52 (86.7)	0.098
URTI	3 (18.8)	28 (46.7)	0.039
LRTI	13 (81.2)	32 (53.3)	
bronchiolitis	0 (0)	7 (11.7)	0.06
bronchopneumonia	1 (6.3)	12 (20)	0.025
pneumonia	12 (75)	13 (21.7)	<0.001
WBC ($\times 10^9$/mL)	12.1 ± 9.1	14.7 ± 8.1	0.904
Neutrophil (%)	76.3 ± 16.4	60.5 ± 20	0.219
BUN (mg/dL), mean (range)	50.0 (5.9–159)	16.4 (1.7–157.7)	0.001
Cr (mg/dL), mean (range)	3.7 (0.56–22.9)	0.4 (0.11–2.9)	<0.001

Abbreviations: URTI = upper respiratory tract infection; LRTI = lower respiratory tract infection; WBC = white blood cells; BUN = blood urea nitrogen; Cr = creatinine; AST = aspartate aminotransferase. [a]p: Statistical significance($p < 0.05$).

clinical presentation compared with a single viral infection.[28,29] Semple et al.[29] reported a ten-fold increase in the relative risk of admission to a pediatric intensive care unit for mechanical ventilation caused by severe bronchiolitis in patients with multiple viral infections. But a different finding was noted for pediatric patients in this study. Physical damage to respiratory cells resulted from viral infection could lead to opportunistic adherence of bacteria.[30] HRV has been historically considered to be a minor pathogen, but with the development of sensitive molecular diagnostics, the virus has been increasingly associated with severe respiratory disease, particularly in children.[12,31] Hospitalized patients who suffered from lower respiratory tract infection might experience viral infection first then followed by bacterial infection.[32]

There were some limitations in this study. First, it is a retrospective study. Inevitably, there are some missing records or laboratory findings in the medical charts. Second,

there might have a selection bias that the specimens were collected from designated divisions. Third, the present study was based on a single center. In addition, the rate of co-infection with any pathogen may interfere the disease presentation. Taken together, these limitations might have potential biases on the results.

In summary, HRV was a common cause of inpatients with respiratory tract infection in Taiwan, particularly in pediatric patients. HRV-A and HRV-C were shown to be predominant in hospitalized patients. Co-infection, with either virus or bacteria, in hospitalized patients was common. Compared with pediatric patients, HRV-infected adult patients were more likely to be associated with a severe respiratory disease entity. The issue whether HRV is a trigger agent, a precipitating agent or even an innocent bystander in these adult patients cannot be figured out clearly. Further studies are needed.

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Transparency declaration

The authors report no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jmii.2018.08.009.