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Original Article

Detection of respiratory viruses in adults with respiratory tract infection using a multiplex PCR assay at a tertiary center

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Abstract *Background:* Respiratory viruses (RVs) are among the most common pathogens for both upper and lower respiratory tract infections (RTIs). However, the viral epidemiology of RV-associated RTIs in adults has long been under-recognized. Through a sensitive molecular assay, it would be possible to have a better understanding of the epidemiology of RV-associated RTIs.
Material and methods: Respiratory tract (RT) specimens from adults hospitalized due to RTIs were tested for RVs, using the multiplex PCR-based Luminex xTAG® Respiratory Viral Panel assay. A total of nineteen RVs, including influenza viruses and non-influenza respiratory viruses (NIRVs) were detected. Positive rates were compared using a chi-square test.
Results: A total of 2292 samples from adult patients hospitalized with RTIs were screened for RVs. The overall positive rate was 22%, with 17.8% samples positive for at least one NIRV. NIRVs had a higher positive rate in non-winter seasons. As many as 12.7% (46/363) of the samples collected through broncho-alveolar lavage and 20.5% (176/859) of the samples collected in ICUs were positive for RVs. Distribution of corona virus (CoV), human metapneumovirus (hMPV) and parainfluenza virus (PIV) demonstrated seasonal variation. Also, temperature was associated with the positive rates of specific viruses, including CoV, respiratory syncytial virus (RSV), hMPV and PIV.
Conclusion: Respiratory viruses, notably NIRVs, were frequently detected in adults

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hospitalized with RTIs. Several RVs were detected with distinctive seasonal variations. A substantial number of RVs were identified in lower RT specimens or from patients admitted to ICU, highlighting their important role in causing severe respiratory infection.

Introduction

Worldwide, respiratory tract infections (RTIs) are a major cause of both mortality and morbidity, and can be categorized into upper and lower RTIs according to the anatomical location that is being infected.[1] In 2015, lower RTIs represented the fourth most common cause of death, after ischemic heart disease, cerebrovascular disease, and chronic obstructive pulmonary disease.[2] Pneumonia, being one of the most important lower RTIs, was reported to have an annual incidence of 24.8 per 10,000 adults and the incidence increased with age.[3] Also, pneumonia is the most common nosocomial infection in the intensive care unit (ICU).[4,5]

Respiratory virus (RV) is a common pathogen for RTI, causing significant mortality and morbidity.[6] Though pathogens responsible for RTI could not be determined in the majority of patients, RVs are more frequently detected than bacteria.[7] In the past, RV-related RTIs were commonly seen and studied in the pediatric population.[8,9] Seasonal variation in RVs throughout the year had also been well-established in the pediatric population.[10–12] The viral epidemiology and prevalence of RTIs in the adult population, however, have long been underestimated and inadequately investigated.[13]

Recognizing the role of RVs on RTIs, especially lower RTIs, is of clinical importance and should not be neglected. RVs had been reported to compose nearly one-third of all hospitalized community-acquired pneumonia, and were actually isolated as frequently as bacterial pathogens in hospital-acquired pneumonia.[14,15] The severity of RV-related lower RTIs ranged from mild disease to those necessitating hospital admission or even mechanical ventilation.[16] Patients with RV-related pneumonia and bacterial pneumonia had comparable rates of mortality.[17] Studies have shown that the prevalence of RV-related RTIs can be over 40% in critically ill patients admitted to ICUs. Nevertheless, less than 50% of the patients with pneumonia admitted to ICUs had been tested for viral pathogens.[18]

The introduction of multiplex PCR assay aids clinical detection for RVs, and provides a better tool to delineate the picture of RV-related RTIs.[19] Multiplex PCR also aids a much earlier detection as compared to conventional diagnostic tests such as viral culture and direct fluorescent antibody test.[20,21] Through applying the multiplex PCR assay platform, this study aimed to investigate the epidemiology of RVs in adults hospitalized with RTIs, including seasonal variations of different viruses and the viral distribution in different inpatients setting.

Material And Methods

Study design

This study was conducted in Taipei Veterans General Hospital (VGHTPE) in Taiwan. We investigated the testing results of RT specimens from patients aged 18 years and older who were hospitalized with suspected RTIs. Tested samples were collected in the emergency department (ED), general wards and ICUs. The sampling methods included nasopharyngeal and throat swabbing for upper RTIs and endotracheal aspiration and broncho-alveolar lavage (BAL) for lower RTIs. Specimens collected from nasopharyngeal swab, throat swab, and endotracheal aspiration were immediately placed into a sterile vial containing viral transport media; as for BAL, the procedure was carried out by a thoracic specialist and the irrigated fluid was collected for further analysis. Samples from the same patient were regarded different when the samples were collected over two weeks apart. Overlap of upper and lower RT samples from the same patient was permitted and was analyzed separately.

Nucleic acids of RT specimens were extracted by an automated extraction system (QIAcube system, Qiagen) and then tested for RVs by the multiplex PCR-based Luminex xTAG® Respiratory Viral Panel (Luminex Molecular Diagnostics Inc) in the Clinical Virology Lab of VGHTPE, according to the manufacturer's instructions. Briefly, the assay comprised a PCR amplification and hybridization step. Signal acquisition presented as median fluorescence intensity was done on the Luminex® 100 IS system. A total of nineteen RVs could be detected by the Luminex xTAG® Respiratory Viral Panel, including influenza (Flu) (influenza A, H1 subtype, H3 subtype, 2009H1N1, influenza B) and non-influenza respiratory viruses (NIRVs) including coronavirus (CoV) (NL63, HKU1, 229E, OC43), rhinovirrus/enterovirus (RhV/EnV), bocavirus (BoV), respiratory syncytial virus (RSV) (RSV subtype A and B), human metapneumovirus (hMPV), parainfluenza virus (PIV) (type 1,2,3,4) and adenovirus (AdV). The presence of each virus generated a signal and was identified as positive by the data analysis software.

Evaluation and analysis

A positive rate was defined as the number of samples positive for RVs divided by the number of all samples collected. We investigated both the overall positive rate and the positive rate excluding influenza. We also investigated the positive rates with respect to different viruses, seasons,

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sampling methods and sampling sites. Data on daily meteorological factors between November 2016 and October 2018 were obtained from the Climatological Data Annual Report published by the Central Weather Bureau in Taiwan. The targeted weather region was Taipei, Taiwan.

Positive rates were compared using a chi-square test. All statistical analyses were performed using IBM SPSS Statistics version 18.0 (IBM Co., Armonk, NY, USA) and Excel (Microsoft Co., Redmond, USA).

Result

Between November 2016 and October 2018, a total of 2292 samples were collected from 2019 patients with RTI screening for RVs. Specimens were collected from ICUs (859 samples, 37.5%), ordinary wards (1129, 49.3%) and ED (304, 13.3%). Among all 2292 samples, 67.6% (1550) of the samples were collected from upper RT through either nasopharyngeal or throat swabbing; while 32.4% (742) were collected from lower RT from either BAL or endotracheal aspiration, while BAL composed 48.9% (363) of the lower RT samples.

Comparison of positive rate

The overall positive rate was 22% (505/2292). Among all the samples, 407 (17.8%) were positive for at least one NIRV; 14 (0.6%) were positive for both flu and NIRV. Among the 2019 patients, 48 (2.4%) patients were simultaneously sampled for both upper and lower RTIs, where only 2 patients were tested positive for both upper and lower RTIs.

Despite the observed monthly variation, the positive rates of NIRV did not differ significantly throughout seasons (18.7%, 18.1%, 20.1% and 14.8% in spring, summer, fall and winter, respectively, $P = 0.127$) (Fig. 1a).

Similar positive rates of RVs were noted in samples from upper and lower RTs (355/1550, 22.9% vs 150/742, 20.2%, $P = 0.146$). The positive rate for NIRVs were also similar between upper and lower RTs (286/1550, 18.5% vs 121/742, 16.3%, $P = 0.209$). In the lower RT, samples from BAL had a lower positive rate compared to endotracheal aspiration (12.7% and 27.4%, respectively, $P < 0.001$). The positive rates for NIRV in upper RT, BAL and endotracheal aspiration samples were 18.5%, 11% and 21.4% ($P = 0.001$) (Fig. 1b). The positive rate for samples collected in ICUs was 20.5% (176/859), which was similar to that of general wards (Fig. 1c).

The overall positive rates of RVs detected were similar between winter and non-winter seasons. However, NIRV had a higher positive rate in non-winter seasons than in winter (18.8% vs 14.8%, $P=0.025$). As for individual viruses, CoV had a higher positive rate in winter (3.6% vs 0.5% $P < 0.001$), while hMPV and PIV had a significantly higher positive rate in non-winter seasons (0.7% vs 3.3%, $P < 0.001$ for hMPV and 2% vs 5.2%, $P = 0.001$ for PIV, respectively. Fig. 1d).

Comparison of seasonality between RVs

RhV and EnV, both belong to the family *Picornaviridae* and were undistinguishable by the mutiplex PCR, were the most commonly detected viruses regardless of season, with no marked seasonal variation observed. PIV was the second most commonly detected NIRV. As for individual viruses, CoV, RSV, hMPV and PIV had significant seasonal variation in positive rates: the positive rates of hMPV and PIV were the highest in spring and gradually decreased from spring to winter; whereas CoV and RSV had the highest detection rate in winter and fall, respectively (Fig. 2a).

In upper RT samples, the seasonal variation in positive rates was similar to that of the overall seasonality (Fig. 2b); whereas, in lower RT samples, only PIV exist seasonal variation in positive rates (Fig. 2c). In addition, PIV was also the only virus having a significant seasonal variation in the positive rate observed in ICU samples, featuring the highest in spring and gradually decreased from spring to winter (Fig. 2d).

Samples collected from lower RT had similar viral distribution with upper RT, with the majority both being RhV/EnV (Fig. 3a). In addition, samples collected from ICUs had similar viral distribution with general wards, with the majority both being also RhV/EnV (Fig. 3b).

Comparison between positive rates and seasonal temperature

Fig. 4 demonstrated the positive rate of individual viruses associated with the maximal and minimal temperatures of each season during the study period. Temperature appeared to have been associated with the positive rates of specific viruses, including CoV, RSV, hMPV and PIV. CoV had the highest positive rate when the temperature reached the lowest. RSV had the highest positive rate in September to November when the temperature was in transition from the highest to the lowest temperature. Considering the similar seasonal variation in positive rates of both hMPV and PIV (Fig. 2a), hMPV combining PIV were plotted together in Fig. 4g which showed the positive rates raised when the temperature was also rising. Other meteorological factors, including precipitation, relative humidity and absolute humidity showed no correlation with positive rates and were thus not included in this study.

Discussion

In this study, we delineated the epidemiologic profile of RV-associated RTIs in adult patients. To our knowledge, this is the first study with a substantial number of samples using a PCR-based method to detect RV infections of adults in Taiwan. Significant variation was noted in individual viruses including CoV, RSV, hMPV and PIV, where temperature seemed to be closely associated with the seasonal variability. Positive rates were similar in samples collected from upper and lower RTs and did not differ regarding seasons and patient locations. Among these viruses, RhV/EnV were the most common virus while PIV was the second most common NIRV regarding sampling sites and patient locations.

Our study highlighted seasonal variation in individual RVs. Consistent with previous reports that CoVs occur primarily in winter,[22–24] we observed that CoVs had the highest positive rate when the temperatures were the lowest. In addition, CoVs were the only NIRV having a higher positive rate in

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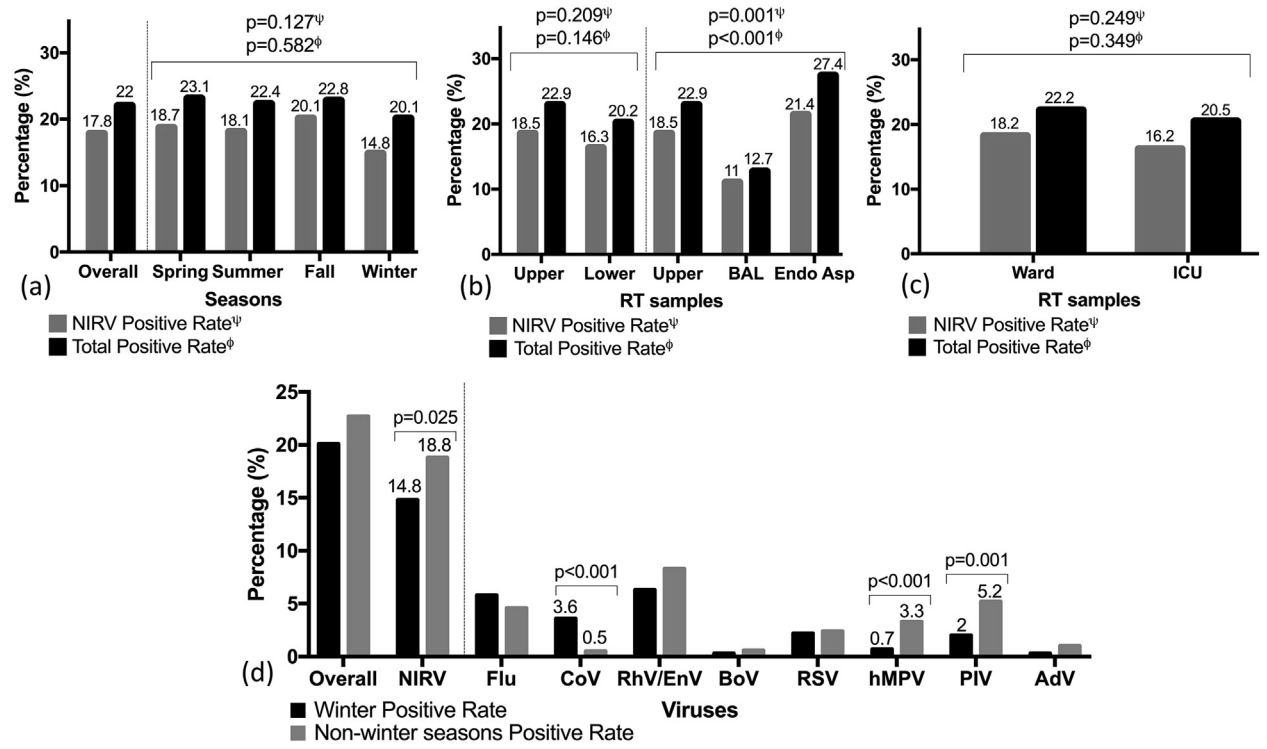

Fig. 1. Comparison of positive rates of samples. (a) Seasonal distribution of positive rates of influenza and other respiratory viruses. (b) Positive rates of samples collected from upper and lower respiratory tracts. (c) Positive rates of samples collected in general wards and ICUs. (d) Positive rates of individual viruses winter and non-winter seasons. NIRV, non-influenza respiratory virus; RT, respiratory tract; BAL, broncho-alveolar lavage; Endo Asp, endotracheal aspiration; ICU, intensive care unit; Flu, influenza; CoV, coronavirus; Rhv/Env, rhinovirus/enterovirus; BoV, bocavirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; PIV, parainfluenza virus; AdV, adenovirus.

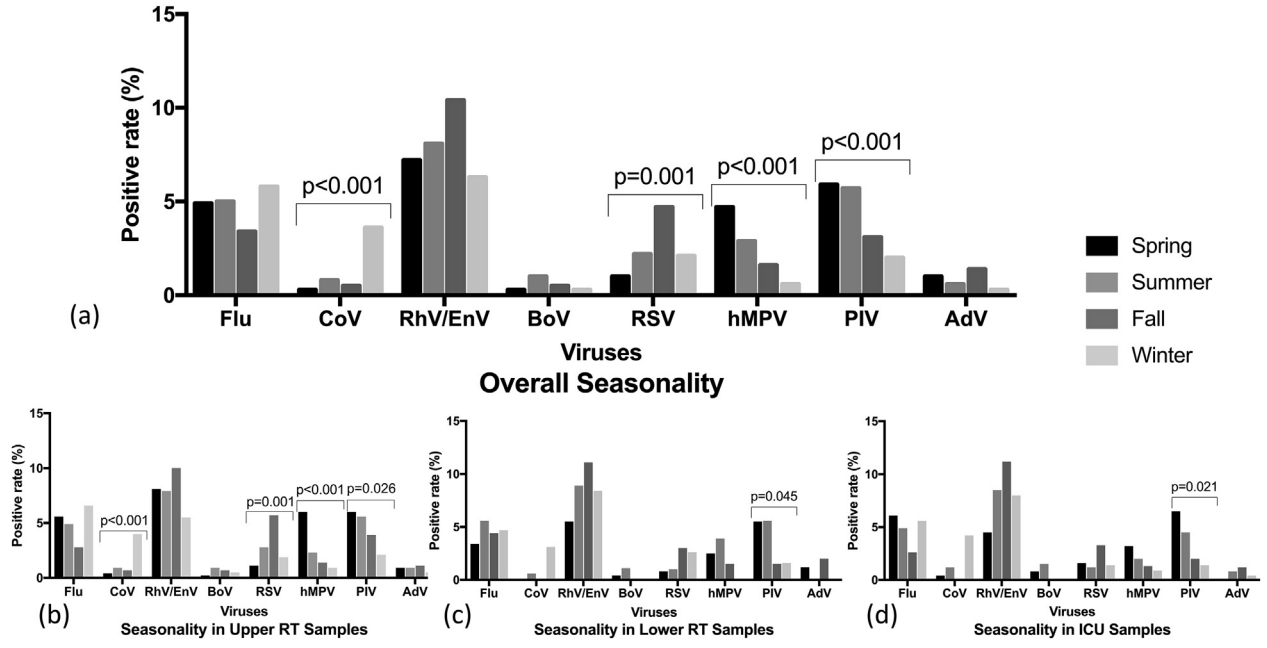

Fig. 2. Seasonal distribution of positive rates of (a) all respiratory viruses, (b) viruses detected in upper respiratory tracts, and (c) viruses detected in lower respiratory tracts.

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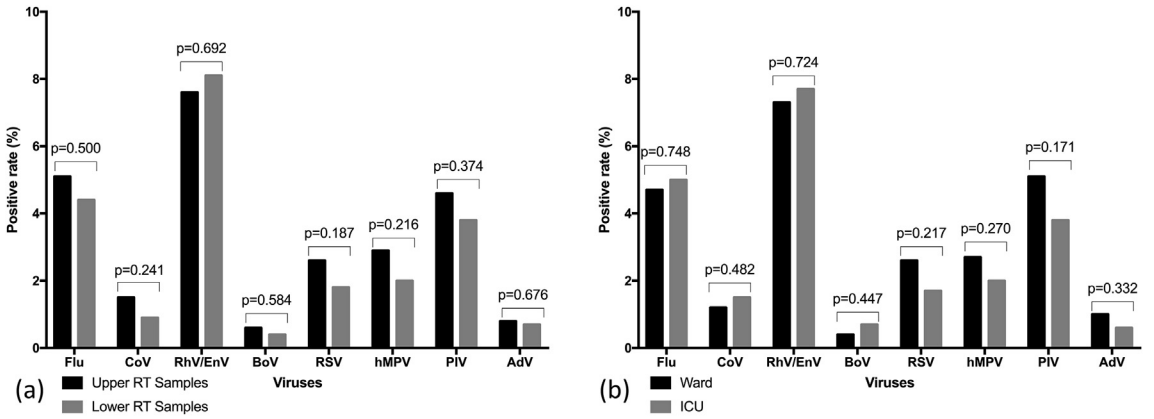

Fig. 3. Comparison of positive rates between (a) samples collected from the upper and lower respiratory tract, and (b) samples collected from ICUs and general wards.

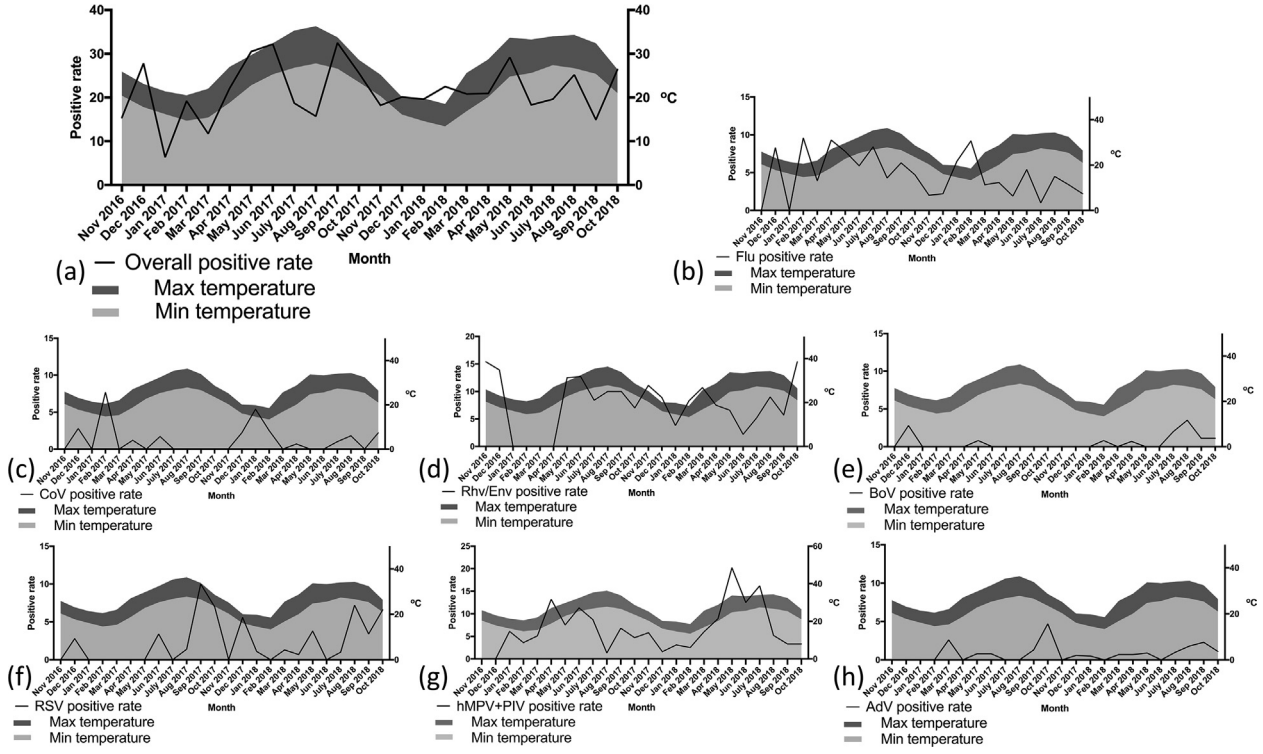

Fig. 4. The association between temperature and positive rates of respiratory viruses.

winter than in non-winter seasons. In a global overview, the activity of RSVs follows the decrement in temperature, while the seasonal variation of hMPVs peaks in spring and early summer in children[25,26]; similar seasonal variation was also observed in adult patients in our study. PIV infections may occur throughout the year, predominating in summer and fall[10,27]; in tropical regions, however, PIVs does not exhibit seasonal variation.[28] In our study, we showed that PIVs dominant in non-winter seasons, especially in spring. Although the correlation between increased mortality rates and respiratory illnesses in winter seasons has been well recognized for years[29]; in non-winter seasons, there were still one third of the patients admitted with lower RTIs having RVs detected by PCR.[30] Together with previously reported findings, our results highlighted the underestimated importance of RV infections in non-winter seasons, given the fact that the positive rate was not lower in non-winter seasons when PIV and hMPV predominated.

Lower RTIs remain a leading burden of both mortality and morbidity worldwide; thus, the importance of detecting pathogens in lower RTs is substantial. Though sampling from nasopharyngeal sites yields a higher detection rate for RVs than BAL, it cannot reflect the viruses in lower RTs.[31] In this study, viruses causing lower RTIs were detected through either BAL and endotracheal aspiration. For detecting viruses in lower RTIs, there were no previous studies comparing the positive rate between BAL and endotracheal aspiration. In our study, in patients needing lower RT sampling for diagnosis,

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over 10% of BAL samples and as many as 27.4% of endotracheal aspiration samples were positive for at least one respiratory virus. Such high positive detection rates indicated that presence of RVs in lower RT is not a rare event in adult patients with pneumonia and suggested that lower RT sampling combined with molecular methods could be a useful tool in diagnosing virus-associated lower RTI.

RV infections are frequently detected in adult patients in ICUs,[32] but have seldom been regarded as a major cause of critical illness.[33] RVs had been found in two-thirds of critical RTI patients in whom exams in BAL did not identify bacteria and RV infection was likely to be the primary cause of death in nearly 50% of these patients.[34] Patients either hospitalized or in ICUs having bacterial RTIs superinfected by RVs also had a poorer survival.[34,35] Although influenza has long been recognized as a cause of severe respiratory infection, NIRVs were also associated with severe illness resulting in ICU admissions 6. In our study, the most common virus related to lower RTI and critical illness was RhV/EnV, with the second most common NIRV being PIV. Although the positive rate of influenza in this study should have been underestimated, the detection of NIRVs in lower RT and ICU patients still highlighted the important role of NIRVs in pneumonia of adult patients.

There were several limitations to our study. First, analyzing the clinical feature was beyond the scope of this study and thus the demographic data, clinical presentation and outcome of each patient were not provided in our results. Still, by including lower RT specimens and specimens collected from ICU, our results suggested that a substantial proportion of the patients were critically ill, which we wished to enlighten future researchers to study the important role of RVs in association with severe respiratory infection. Second, the use of multiplex PCR for detecting RVs was frequently preceded by a negative result of rapid influenza diagnostic test; thus, the positive rate of influenza and the coinfection rate of flu and NIRV in our study were expected to be underestimated. Third, the retrospective design of the study may have led to an inevitable selection bias. For instance, multiplex PCR was tested in patients with presumably more severe diseases in our hospital; RV infection in patients with mild diseases may be underestimated in this study.

In conclusion, respiratory viruses, notably NIRVs, were frequently detected in adult patients with RTIs. Several RVs including CoV, RSV, hMPV and PIV were detected with distinctive seasonal variations. A substantial number of RVs were identified in lower RT specimens or from patients admitted to ICU, highlighting their important role in causing severe respiratory infection. Further studies on the clinical presentation and outcome of patients are warranted.

Transparency declaration

The authors declare that they have no competing interests.

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