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Detection of respiratory pathogens by application of multiplex PCR panel during early period of COVID-19 pandemic in a tertiary hospital in Central Taiwan

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KEYWORDS
Respiratory tract infection; Virus; Atypical pathogen; Multiplex PCR

Abstract  Background: Respiratory tract infections (RTIs) represent a major cause of clinical visits worldwide. Viral epidemiology of RTIs in adults has been less studied compared to children. FilmArray respiratory panel (FA-RP), a multiplex, real time polymerase chain reaction method can simultaneously detect the nucleic acids of multiple pathogens. The purpose of this study is to analyze the epidemiology and clinical presentations of an RTI cohort.

Methods: This retrospective cohort study was conducted at China Medical University Hospital (CMUH) and China Medical University Children’s Hospital (CMUCH), from January 2020 to June 2020. The FA-RP results were collected and analyzed according to upper versus lower RTIs.

Results: Among 253 respiratory samples tested, 135 (53.4%) were from adults and 118 (46.6%) from children. A total positive rate of 33.9% (86/253) was found, with 21.48% (29/135) in adults and 48.31% (57/118) in children. Human rhinovirus/Enterovirus (HRV/EV) was detected in most of the age groups and was more common in URIs. HRV/EV was found as a frequent co-detection virus. Among children, HRV/EV was the most detected pathogen of URIs, while the most predominant pathogen in LRIs was Mycoplasma pneumoniae.

Conclusions: FA-RP has the potential to improve the detection rate of respiratory pathogens. The positive rate of FA-RP was higher in children compared to adults, which likely corresponds to the higher infection rate in children.
Materials and methods

This observational retrospective study was conducted at China Medical University Hospital (CMUH) and the adjacent China Medical University Children’s Hospital (CMUCH), a tertiary hospital in Taichung, Taiwan. From January 1, 2020 to June 30, 2020, the early period of the COVID-19 pandemic in Taiwan, we investigated the testing results of nasopharyngeal specimens from patients who visited our hospitals. Those who had fever or symptoms of RTIs were included in the study; otherwise, patients were excluded. The tested samples were collected from the out-patient department (OPD), emergency department (ED), general wards, and intensive care units (ICUs) (Supplementary Fig. 1). Patients who had symptoms or signs of lower respiratory tract infections, as proven by chest X-ray, were diagnosed by clinicians as lower respiratory tract infections, otherwise, patients were diagnosed as upper respiratory tract infections. All the specimens were tested via BioFire FilmArray® Respiratory Panel (FA-RP) version 2.0, which can detect 17 respiratory viruses and four bacterial targets, including influenza A virus (H1/2009, H1, H3, non-subtyped), influenza B virus, parainfluenza virus (PIV) (type 1,2,3,4), adenovirus (ADV), coronaviruses (HCoV) (OC43, NL63, 229E, HKU1), respiratory syncytial virus (RSV), human metapneumovirus (Hmpv), human rhinovirus/enterovirus (HRV), Bordetella pertussis, Bordetella parapertussis, Chlamydia pneumoniae, and M. pneumoniae in a multiplex polymerase chain reaction (PCR). The FA-RP provided an automated extraction of nucleic acids, reverse-transcription, nucleic acid amplification technologies, and melting curve analysis of amplified products. Conventional diagnostic tests for the identification of pathogens of RTIs, including culture-based and immunological methods, are widely used. However, these methods are time-consuming and cumbersome. New diagnostic methods, such as multiplex nucleic acid amplification tests (NAAT) and molecular point-of-care testing (POCT) are faster and highly sensitive and have the potential to improve the detection rate of respiratory pathogens. One study reported a 30%–50% increase in the diagnosis of respiratory viruses with the use of a multiplexed molecular test compared to culture-based and immunological methods. The accurate and rapid detection of causative pathogens plays a significant role in selecting appropriate therapy, minimizing therapy costs, and controlling the disease. In December 2019, infections with the new pandemic pathogen Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), emerged in Wuhan, China, resulting in a global outbreak by the following year. Some pandemic-prevention policies, including border controls and mandatory quarantines, had been implemented in Taiwan. People have also been required to wear face masks when taking public transportation and to reduce participation in cluster activities. Thus, the viral epidemiology of RTIs in Taiwan seemed to undergo huge changes in 2020.

In this study, we investigate the epidemiology of respiratory viruses identified in the respiratory specimens of patients with respiratory tract infections by using the BioFire FilmArray® Respiratory Panel (FA-RP). Viral distributions in different age groups and the locations of RTIs are discussed separately.

Ethics

This study was approved by the Institutional Review Board of CMUH (CMUH19-REC3-108), with a waiver regarding informed consent. All methods of this study were performed in accordance with the relevant guidelines and regulations.

Statistical analysis

The data in this study were analyzed by the chi-square test, Fisher’s exact tests and Kruskal–Wallis H test. All statistical
analyses were performed using IBM SPSS Statistics version 25.0 (IBM Co., Armonk, NY, USA).

Results

Spectrum of respiratory pathogens detected

A total of 253 respiratory samples were collected, among which 104 pathogens were detected. Among 253 respiratory samples tested, 118/253 (46.6%) were from children and 135/253 (53.4%) were from adults. The numbers of each age group are evenly distributed, apart from the age group between 18 and 44 years. The percentage of patients with LRIs (56.9%) is greater than that of patients with URIs (36.0%). The overall positive rate is 33.9% (86/253), which is higher in the children’s group (57/118, 48.31%) compared to that in the adult group (29/135, 21.48%). The positive rate among 2–4 age group is the highest (22/28, 78.57%), followed by the age groups of ≤2 years (20/29, 68.97%) and 18–44 years (19/67, 28.36%). The negative samples were mostly attributed to patients in the older age group (Table 1).

The number of positive samples by specific viral agent and month of diagnosis are shown in Fig. 1, while the distribution of pathogens within each age group are presented in Supplementary Fig. 2. HRV/EV were the most prevalent organisms detected (50 positive samples, 47.61% of the total), which prevailed throughout all the months assessed. The second most prevalent organisms identified were ADV (18 isolates, 17.14% of the positive samples). FLU was detected only from January to March, while M. pneumoniae was detected only in February to April (Supplementary Table 1). The results vary between children and adults, as shown in Table 2. In the children’s group, HRV/EV were the most common isolated pathogens, followed by ADV and M. pneumoniae. In the adult group, HRV/EV were the most common, followed by FLU, ADV, and M. pneumoniae.

Multiple detections

Detection of more than one respiratory pathogen was found in 17/86 (19.77%) of the positive samples, with a higher co-detection rate in the children’s group (14/57, 24.56%) than in the adult group (3/29, 10.34%). However, this was not found to be statistically significant with a p value of 0.156. Fifteen samples detected two respiratory pathogens, one sample detected three respiratory pathogens, and one sample detected four respiratory pathogens. HRV/EV is found in most of the multiple isolations, making it the most frequent isolated pathogen. The most common combinations of isolated pathogens are HRV/EV and ADV (total 6/17, 35.29%), as shown in Supplementary Table 2.

Table 1  Baseline characteristics of 253 patients with positive versus negative samples.

| Variable                          | Total, n = 253 | Positive, n = 86 | Negative, n = 167 | p value |
|-----------------------------------|----------------|------------------|-------------------|---------|
| Gender, male                      | 134 (53.0)     | 42 (48.8)        | 92 (55.1)         | 0.345   |
| Age groups                        |                |                  |                   |         |
| <18 years                         | 118 (46.6)     | 57 (66.3)        | 61 (36.5)         | <0.001  |
| <2 years                          | 29 (11.5)      | 20 (23.3)        | 9 (5.4)           |         |
| 2–4 years                         | 28 (11.1)      | 22 (25.6)        | 6 (3.6)           |         |
| 5–9 years                         | 29 (11.5)      | 8 (9.3)          | 21 (12.6)         |         |
| 10–17 years                       | 32 (12.6)      | 7 (8.1)          | 25 (15.0)         |         |
| ≥18 years                         | 135 (53.4)     | 29 (33.7)        | 106 (63.5)        |         |
| 18–44 years                       | 67 (26.5)      | 19 (22.1)        | 48 (28.7)         |         |
| 45–64 years                       | 36 (14.2)      | 5 (5.8)          | 31 (18.6)         |         |
| ≥65 years                         | 32 (12.6)      | 3 (3.5)          | 29 (17.4)         |         |
| Clinical manifestations           |                |                  |                   |         |
| Fever without other respiratory tract symptoms | 44 (32.8) | 7 (8.1) | 37 (22.2) | 0.005 |
| Clinical diagnosis                |                |                  |                   |         |
| Respiratory tract infections      | 235 (92.9)     | 84 (97.7)        | 151 (90.4)        | 0.038   |
| URIs                              | 91 (36.0)      | 35 (40.7)        | 56 (33.5)         | 0.490   |
| LRIs                              | 144 (56.9)     | 49 (57.0)        | 95 (56.9)         | 0.490   |
| Non respiratory tract infections  | 18 (7.1)       | 2 (2.3)          | 16 (9.6)          | 0.038   |

Abbreviation: URIs = upper respiratory tract infections; LRIs = lower respiratory tract infection.

Data are presented as case number (percentages). Statistical significance is indicated in bold.
Respiratory infections are a common cause of morbidity worldwide. In particular, viruses and atypical pathogens are major causes of pediatric RTIs. Multiplex nucleic acid-based molecular detection techniques have the potential to improve the detection rate of respiratory pathogens, and reduce antibiotic use. With the increasing availability of multiplex PCR panels, some viruses, such as HMPV, human coronavirus, and HRV, which have previously been difficult to cultivate, are now being detected frequently using the NAAT methods.

In our study, 253 nasopharyngeal specimens were collected and analyzed with FA-PR over a period of six months, during the COVID-19 pandemic. The overall results revealed a positive rate of 33.9%, in agreement with the range of positive rates (33.3%–65.2%) reported in other studies. With the lowest positive rate of 33.3% reported in a previous study performed on adults. Likewise, our relatively low overall positive rate could be explained by our wide range in age groups that included both children and adults. HRV/EV and ADV are the most common viruses detected in previous studies prior to the COVID-19 pandemic. During the early period of the pandemic, HRV/EV remained the most frequently detected virus in the current study, followed by ADV. HRV/EV and ADV are non-enveloped viruses that are resistant to alcohol-based hand sanitizers, which have been emphasized as important tools in preventing COVID-19 infections. Similar to previous studies, we observed a higher detection rate of respiratory viruses in children younger than 5 years (42/57, 73.7%). The higher detection rate among young

| Pathogens                      | <18 years Positive (n = 57) | Co-detected (14/57, 24.6%) | ≥18 years Positive (n = 29) | Co-detected (3/29, 10.3%) | p value |
|-------------------------------|-----------------------------|-----------------------------|----------------------------|--------------------------|---------|
| Human rhinovirus/enterovirus  | 37 (51.4)                   | (10/37, 27.0%)              | 13 (39.4)                  | (2/13, 15.4%)           |         |
| Adenovirus                    | 14 (19.4)                   | (9/14, 64.3%)               | 4 (12.1)                   | (1/4, 25%)              |         |
| Mycoplasma pneumoniae         | 9 (12.5)                    | (2/9, 22.2%)                | 4 (12.1)                   | 0                       |         |
| Parainfluenza virus           | 8 (11.1)                    | (5/8, 62.5%)                | 1 (3.0)                    | (1/1, 100%)             |         |
| RSV                           | 2 (2.8)                     | (2/2, 100%)                 | 2 (6.1)                    | (1/2, 50%)              |         |
| Coronavirus                   | 1 (1.4)                     | 0                           | 3 (9.1)                    | (1/3, 33.3%)            |         |
| Human metapneumovirus         | 1 (1.4)                     | (1/1, 100%)                 | 0                          | 0                       |         |
| Influenza virus               | 0                           | 0                           | 6 (18.2)                   | (1/6, 16.7%)            |         |

Detection of more than one respiratory pathogen was found in 17/86 (19.77%) of the positive samples, with a higher co-detection rate in the children’s group (14/57, 24.56%) than in the adult group (3/29, 10.34%). However, this was not found to be statistically significant with a p value = 0.156.

**Discussion**

Respiratory infections are a common cause of morbidity worldwide. In particular, viruses and atypical pathogens are major causes of pediatric RTIs. Multiplex nucleic acid-based molecular detection techniques have the potential to improve the detection rate of respiratory pathogens, and reduce antibiotic use. With the increasing availability of multiplex PCR panels, some viruses, such as HMPV, human coronavirus, and HRV, which have previously been difficult to cultivate, are now being detected frequently using the NAAT methods.

In our study, 253 nasopharyngeal specimens were collected and analyzed with FA-PR over a period of six months, during the COVID-19 pandemic. The overall results revealed a positive rate of 33.9%, in agreement with the range of positive rates (33.3%–65.2%) reported in other studies. With the lowest positive rate of 33.3% reported in a previous study performed on adults. Likewise, our relatively low overall positive rate could be explained by our wide range in age groups that included both children and adults. HRV/EV and FLU are the most common viruses detected in previous studies prior to the COVID-19 pandemic. During the early period of the pandemic, HRV/EV remained the most frequently detected virus in the current study, followed by ADV. HRV/EV and ADV are non-enveloped viruses that are resistant to alcohol-based hand sanitizers, which have been emphasized as important tools in preventing COVID-19 infections. Similar to previous studies, we observed a higher detection rate of respiratory viruses in children younger than 5 years (42/57, 73.7%). The higher detection rate among young
In the children group, the difference in causative pathogens amongst URIs versus LRIs was found to be statistically significant with a value of 0.005. This observation highlights the value of FA-RP testing in patients presenting with both fever and respiratory symptoms.

In our study, results showed that HRV/EV was the most commonly identified virus in almost all age groups, especially infected young children, in accordance with previously published data. HRV is difficult to culture, while multiplex PCR panel allows the rapid detection of HRV. Human Rhinoviruses (HRVs) are small (15 nm), non-enveloped viruses containing a single-stranded RNA genome. There are more than 150 different HRV types with almost no cross-protection, thus explaining the frequency of HRV infections. The transmission routes of HRVs via aerosols of respiratory droplets and hand-related transmissions have been confirmed in past works. However, Leung et al. demonstrated no significant differences between the detection of viruses with or without face masks, both in respiratory droplets and in aerosols. Furthermore, the median duration of virus shedding is 11 days for rhinovirus in immunocompetent children. Therefore, recurrent or overlapping infections caused by different genotypes of HRVs are frequent. The above reasons may explain the predominant detected respiratory pathogens.

Influenza virus, previously thought to be the most common pathogen, only appeared in 2.3% of our positive samples. This result is consistent with the statistical data obtained from the Taiwan Centers for Disease Control in the same period of 2020. During the year of the COVID-19 pandemic, wearing face masks became a daily routine for people living in Taiwan. We thus observed a decline in the proportion of viral infections afterward. Although a study demonstrated that surgical masks can efficaciously reduce the spread of FLU particles within respiratory droplets into the environment, a Cochrane study demonstrated no clear reduction in respiratory viral infections from using surgical masks during seasonal influenza. Adenovirus, a non-enveloped virus, represents the second most common pathogen in children’s group. In our study, the age group younger than 5 years seem to be at a higher risk of getting infected.

*M. pneumoniae* accounts for 10–30% of community-acquired pneumonia in children in Taiwan, with a high prevalence amongst school-aged children and adolescents. When infected with *M. pneumoniae*, children younger than 3 years tend to develop upper airway infections, whereas children between 5 and 20 years tend to develop acute bronchitis and pneumonia. In our study, *M. pneumoniae* had a detection rate of 12% and prevailed in the 5–9 year age group, with all patients suffering from LRIs. Previous studies have reported varying detection rates of *M. pneumoniae*: 1.2% (adults and children in Greece), 1.7% (adults and children in U.S.), 7.1% (adults in Shanghai, China), to 12.8% (children in Rome, Italy), which might be attributed to the difference in collection sites.

Co-infection has been found in 31–51.8% of positive respiratory samples in previous studies. However, in our study, a lower rate of 19.77% co-infection was detected, with a higher rate of co-infection noted in children (24.56%). The largest proportion of co-detected pathogens was HRV/EV. Combinations of HRV/ADV and HRV/RSV have been previously found as the most frequent co-infection viruses. However, one study suggested that the presence of RSV reduces the probability of HRV infection. In our study, the most common combination of co-infections occurred with HRV/EV and adenovirus (35.7% in the children’s group), and the combination of HRV/EV and RSV was found in one child presenting with lower RTI. HRV causes clinical symptoms in patients with co-infections, rather than being just an incidental finding. Although the high sensitivity of PCR allows the detection of minute amounts of viral nucleic acids, questions remain concerning the clinical relevance of positive test results.

### Table 3 Relationship between detected pathogens and the type of respiratory infection.

| Pathogens                        | <18 years (n = 57) | ≥18 years (n = 29) | All age groups (n = 86) |
|----------------------------------|--------------------|-------------------|-----------------------|
|                                  | URIs (n = 27)      | LRIs (n = 30)     | URIs (n = 7)          | LRIs (n = 22) | All age groups (n = 86) |
| Human rhinovirus/enterovirus     | 24 (88.9)          | 13 (43.3)         | 5 (71.4)              | 8 (36.4)      | 50 (58.1)               |
| Adenovirus                       | 8 (29.6)           | 6 (20)            | 1 (14.3)              | 3 (13.6)      | 18 (20.9)               |
| *Mycoplasma pneumoniae*          | 0                  | 9 (30)            | 0                     | 4 (18.2)      | 13 (15.1)               |
| Parainfluenza virus              | 3 (11.1)           | 5 (16.7)          | 1 (14.3)              | 1 (4.5)       | 9 (10.5)                |
| RSV                              | 0                  | 2 (6.7)           | 0                     | 2 (9.1)       | 4 (4.7)                 |
| Coronavirus                       | 0                  | 1 (3.3)           | 1 (14.3)              | 2 (9.1)       | 4 (4.7)                 |
| Human metapneumovirus            | 0                  | 1 (3.3)           | 0                     | 0             | 1 (1.2)                 |
| Influenza virus                  | 0                  | 0                 | 0                     | 6 (27.3)      | 6 (7.0)                 |

* In the children group, the difference in causative pathogens amongst URIs versus LRIs was found to be statistically significant with a p value = 0.035.

* No statistical significance was found in the adult age group with regards to causative pathogens of URIs versus LRIs (p value = 0.258).

Abbreviations: URIs = lower respiratory tract infection; RSV = Respiratory syncytial virus; URIs = upper respiratory tract infection.

Data are presented as case number (percentages).
infectious shedding or asymptomatic colonization, and may not represent acute infection.\textsuperscript{33} Viral cultures must be performed to document long durations of virus shedding. However, concomitant viral culture with PCR is not performed in co-detection patients to document this idea. In contrast to RSV, hMPV, and ADV, viruses such as HRV and ADV are not performed to document long durations of virus shedding. HRV may also lead to more severe respiratory tract symptoms, such as pneumonia, bronchiolitis, and asthma.\textsuperscript{34} In our study, seven children infected with HRV suffered from dyspnea.

This study has some limitations that should be addressed in future works. First, our sample size is based on specimens collected from a single location in the period of six months. This may limit the general applicability of the findings and viral epidemiology may also be affected by seasonal changes. Second, all patients received FA-RP only. Viral cultures were only performed in a handful of patients, thus making it difficult to determine if the pathogen detected on FA-RP reflects an acute or previous infection. The positive rate of virus infection and co-infection rate between pathogens in our study may be overestimated since post-infectious shedding or asymptomatic colonization could not be accounted for. The detection rate for LRIs may also be underestimated since FA-RP is based solely on nasopharyngeal specimens. Third, HRV/EV both belong to the Picornaviridae family, which could not be differentiated via FA-RP. However, clinical presentations of EV infections, such as hand-foot-mouth and herpangina differed from HRV infections. Finally, SARS-CoV-2 could not be detected by FA-RP version 2.0. The advantage of using FA-RP version 2.0 for clinicians is the rapid detection of multiple respiratory pathogens other than SARS-CoV-2. Detection of SARS-CoV-2 was made available on the upgraded version 2.1 of FA-RP. However, FA-RP version 2.1 was not available in our hospital during the study period.

In conclusion, FA-RP has the potential to improve the detection rate of respiratory pathogens. The clinical manifestations and the affected locations varied between different pathogens. The adequate epidemiological surveillance of respiratory virus infections may improve the diagnosis, thus leading to appropriate treatment and therapy.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmiit.2021.09.011.