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Original Article

Unveiling the dynamics of respiratory infections revealed by multiplex PCR testing during the COVID-19 pandemic in Taiwan, 2020—2023

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KEYWORDS
BioFire Respiratory Panel 2.1 (RP 2.1);
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Respiratory syncytial virus;
Vaccination strategies

Abstract *Background:* The emergence of SARS-CoV-2 in late 2019 sparked the global COVID-19 pandemic, leading to varied vaccine policies worldwide. The evolving patterns of respiratory pathogens, aside from SARS-CoV-2, during the pandemic have had a significant impact on the development of vaccine strategies.
Methods: This study explores the landscape of respiratory pathogens, encompassing SARS-CoV-2, respiratory syncytial virus (RSV), and influenza viruses, through a retrospective analysis of data obtained from the BioFire Respiratory Panel 2.1 (RP 2.1) at China Medical University Hospital (Taichung, Taiwan) spanning from January 2020 to November 2023.
Results: Among the 7950 respiratory samples studied, pediatric cases exhibited higher

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positivity (64.9%, 2488/3835) and mixed detection rates (43.8%, 1090/2488) than adults. Annual mixed detection rates increased (27.9—48%). Prevalence analysis revealed diverse patterns across age groups, with higher rates in pediatrics. Notably, human rhinovirus/enterovirus predominated (48.1%). Mixed detection illustrated viral co-detections, notably with parainfluenza viruses and adenovirus. Government policies and pandemic dynamics influenced infection patterns, with RSV resurgence after May 2022. Age-specific RSV detection demonstrated a shift, influencing vaccine considerations. Amid global vaccine initiatives, RSV's increasing trend in adults warrants attention.

Conclusions: This comprehensive analysis emphasizes the importance of multiplex PCR testing in shaping targeted vaccination strategies during evolving respiratory pathogen landscapes.

Introduction

In late 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China, unveiling the viral pathogen responsible for the onset of coronavirus disease 2019 (COVID-19) and triggering a global health crisis.[1] Despite the development of effective vaccines that induce the production of protective antibodies, various countries have begun formulating diverse vaccine policies due to this pandemic, highlighting the importance of vaccine efficacy.[2,3] This emphasis requires a detailed examination of regional dissemination patterns, encompassing not only SARS-CoV-2 but also respiratory pathogens like respiratory syncytial virus (RSV), influenza viruses, and enterovirus.

The evolving trend towards the continuous integration of rapid sample-to-answer multiplex polymerase chain reaction (PCR) testing for respiratory viruses in hospital settings, potentially replacing current reliance on rapid antigen tests or viral culture. The efficiency of PCR testing optimizes the screening process for various viral pathogens, ensuring a comprehensive, resource-efficient diagnostic approach.[4] The BioFire® Respiratory Panel 2.1 (BioFire RP2.1), which previously obtained FDA Emergency Use Authorization (EUA), is a multiplex PCR panel, enabling simultaneous testing for SARS-CoV-2 and other significant respiratory pathogens including influenza, RSV and adenovirus and so on.[5,6]

Our aim is to enhance the understanding of the diversity and dynamic patterns of upper respiratory tract infections through a retrospective analysis of multiplex PCR data from a tertiary medical center in Taiwan. This analysis spans the period from 2020 to 2023, encompassing the initial stages of COVID-19 to the present. This retrospective study also aims to provide insights that can contribute to the establishment of national vaccine policies and align with international vaccine strategies.[13]

Materials and methods

Study design

We conducted a retrospective analysis of patients who sought medical attention at a tertiary medical center in central Taiwan, from January 2020 to November 2023 due to symptoms of respiratory tract infection. The inclusion criteria covered outpatient, emergency, and inpatient cases, which included patients in both the wards and intensive care units. We used laboratory order codes to include all patients who underwent the BioFire® FilmArray Respiratory PCR Panel 2.0 and 2.1. This multiplex PCR test, utilizing the BioFire® FilmArray Respiratory PCR Panel 2.1 (BioFire RP2.1, BioFire Diagnostics, bioMérieux, Marcy l'Etoile, France), screened for SARS-CoV-2 and other respiratory pathogens. Prior to July 1, 2021, we employed FilmArray system version 2.0, which lacked inclusion of SARS-CoV-2 due to Taiwan Food and Drug Administration regulations.

All patients who underwent this multiplex PCR test did so based on the clinical decisions of their physicians and their symptoms of respiratory tract infection, with the costs covered by the National Health Care Insurance. Due to hospital policy and the prevailing societal concerns during the COVID-19 period, any febrile episode in the pediatric group prompted testing. In the adult group, testing was conducted for those with fever accompanied by respiratory symptoms such as cough, rhinorrhea, and sore throat, particularly for immunocompromised patients, such as those who had undergone organ transplantation, were receiving immunosuppressants, or were HIV-positive. The institutional review board (IRB) approved the collection of data from each patient. This study was approved by the IRB of the CMUH (IRB number: CMUH112-REC3-041).

BioFire® FilmArray respiratory PCR panel 2.1

The sample provided for the FilmArray Respiratory PCR Panel 2.1 was extracted from nasopharyngeal or oropharyngeal swabs, following both the instructions of the manufacturer and medical standards. The panel enables simultaneous testing for 18 viruses, including SARS-CoV-2, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, influenza A, influenza virus A/H1, influenza virus A/H1-2009, influenza virus A/H3, influenza virus B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, adenovirus, human metapneumovirus, human rhinovirus/enterovirus, and RSV.

Additionally, it allows for the testing of four bacterial pathogens: *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

Characteristics of patients

We also conducted a review of patients' medical records, collecting data such as their gender, age, testing location (emergency department, inpatient, and outpatient) and identified pathogens. All patients who underwent PCR testing were included, and no one was excluded during the study period. We categorized all participants into either a pediatric or adult group. Individuals aged 18 years or older were assigned to the adult group, while those under 18 years were placed in the pediatric group. To differentiate respiratory pathogens among distinct age groups, we categorized the participants into ten subgroups based on their age ranges (0—1 year, 1—2 years, 2—4, 5—9, 10—17, 18—24, 25—34, 35—54, 55—74, and 75 or older).[7,8,9] We also performed subgroup analysis in accordance with the Advisory Committee on Immunization Practices (ACIP) 's RSV Vaccine recommendations, categorizing participants into three groups: Infants (age $\leq$2 years), Young children (age $>$2 - $\leq$5 years), School children (age $>$6-$\leq$17 years), Adult (age $\geq$18-$\leq$59 years) and Elders (aged 60 years and older).[10,11] Mixed detection referred to more than one pathogen detected from a single sample. Mixed detection rate was defined by the number of mixed-detected samples divided by the tested positive samples. The frequency of mixed detection was determined by identifying and counting pairs of pathogens that were simultaneously detected in each tested sample, meaning that for each sample, the presence of multiple pathogens was recorded and the occurrence of specific pairs of pathogens appearing together was measured to define how often such mixed detections occurred.

Statistical analyses

The statistical analyses were performed with MedCal®, version 20.015. A *p*-value of $<$0.05 was considered to be indicative of a statistically significant result. Continuous variables are presented as the median and interquartile range (IQR), and categorical variables are shown as n (%). The Chi-square test is employed for categorical variable associations between pediatric and adult groups. All collected data were anonymized.

Results

Study population and characteristics

In this retrospective study, a total of 7950 respiratory samples were examined (Table 1), with 3835 from the

Table 1 Characteristics of patients who underwent the BioFire® Respiratory Panel 2.1 testing from January 2020 to November 2023.

Characteristics	No. (%) of patients			
	Overall (n = 7950)	Pediatric (n = 3835)[a]	Adult (n = 4115)	*P* value[b]
Age, median (interquartile range), years	13.3 (2—10)	3.5 (1—4)	51.3 (34—66)	
Male	4176 (52.5)	2102 (54.8)	2074 (50.4)	0.479
Positive result	3133 (39.4)	2488 (64.9)	645 (15.7)	<0.0001
No. of samples with multiple pathogens (% of samples with a positive result)	1201 (38.3)	1090 (43.8)	111 (17.2)	<0.0001
Patient source of clinical samples with a positive result				
Inpatients (n = 3422)[c]	1607 (20.2)	1304 (34.0)	303 (7.4)	<0.0001
Emergency department (n = 3582)	969 (12.2)	770 (20.1)	199 (4.8)	0.0014
Outpatients (n = 946)	557 (7.0)	414 (10.8)	143 (3.5)	0.027
No. of pathogens detected in a clinical sample				
1	1932 (24.3)	1398 (36.5)	534 (13.0)	
2	809 (10.2)	717 (18.7)	92 (2.2)	
3	268 (3.4)	255 (6.6)	13 (0.3)	
4	79 (1.0)	74 (1.9)	5 (0.1)	
5	28 (0.4)	27 (0.7)	1 (0.02)	
6	11 (0.1)	11 (0.3)		
7	3 (0.04)	3 (0.1)		
8	2 (0.03)	2 (0.1)		
9	0 (0.0)	0 (0.0)		
10	1 (0.01)	1 (0.03)		

[a] Individuals under 18 years of age (age <18 years).
[b] *P* values in boldface indicate that the difference is statistically significant (<0.05).
[c] The numbers in the brackets indicate the number of patients from each source who were tested with BioFire® Respiratory Panel 2.1.

pediatric population and 4115 from adults. Median age was 13.3 years old. There was no gender difference between the two groups, with males constituting 54.8% in the pediatric population and 50.4% in adults.

Performance of multiplex PCR

Among all the tested samples, the overall positivity rate of BioFire RP2.1 was 39.4% (n = 3133), and notably, the pediatric group exhibited a significantly higher positivity rate (64.9%, n = 2488) compared to adults (15.7%, n = 645) with statistical significance ($p < 0.0001$). The pediatric group demonstrated a higher propensity for multiple pathogens detection, with a rate of 43.8% (n = 1090), compared to adults with a rate of 17.2% (n = 111) ($p < 0.0001$). An annual increase in mixed detection rates was observed from 2020 to 2023, with rates of 81 (27.9%), 131 (23.2%), 349 (36.9%), and 640 (48%), respectively. Furthermore, the pediatric group demonstrated the ability to detect up to 10 pathogens in a single sample, with pathogen numbers ranging from 6 to 8, a feature not observed in the adult group. Specifically, the pediatric group exhibited the following results: 6 pathogens (n = 11, 0.3%), 7 pathogens (n = 3, 0.1%), and 8 pathogens (n = 2, 0.1%). All instances with ≥6 pathogens detected in single sample occurred exclusively in 2023. Table 2 provides a detailed list of the pathogens detected in each individual sample. In contrast, the adult group showed a maximum of 5 pathogens detected (n = 1, 0.02%).

Prevalence of pathogen by age group

We also observed the prevalence (Table 3) of each age group among positive samples detected by the BioFire Respiratory Panel 2.1. The predominant organisms identified in this study included human rhinovirus/enterovirus (n = 1,508, 19.0%), RSV (n = 633, 8.0%), adenovirus (n = 574, 7.2%), SARS-CoV-2 (n = 484, 6.1%), and parainfluenza 3 (n = 461, 5.8%). Among these five common viral detections, the prevalence rates were higher in the pediatric group compared to adults. Viral detections in the pediatric group also exhibited greater diversity, while adults showed a notable prevalence of SARS-CoV-2 and human rhinovirus/enterovirus detections. If taken together (Fig. 1), among the overall 7950 samples, BioFire RP2.1 revealed a negative rate of 61.6% (n = 4817) for the studied respiratory pathogens. The incidence rate of all 3133 positively tested samples, viral detections accounted for more than 99% samples, with human rhinovirus/enterovirus (48.1%) being the most common, followed by parainfluenza (26.1%), RSV (20.2%), adenovirus (18.3%), and SARS-CoV-2 (15.4%). Although *M. pneumoniae* was thought to be a common pathogen in respiratory tract infection, the positive rate of detection was only 0.23% (18/7950).

Frequency of mixed detection by pathogens

Regarding multiple pathogens detection (Fig. 2), we present the frequency of mixed detection or co-detection by two viral pathogens. Human rhinovirus/enterovirus is most commonly detected in conjunction with parainfluenza (n = 366), followed by adenovirus (n = 243) and RSV (n = 228). Additionally, during the COVID-19 pandemic era, the most frequent co-detection with SARS-CoV-2 involves human rhinovirus/enterovirus (n = 109), followed by influenza (n = 92).

Table 2 Presence of ≥6 pathogens in a sample detected by the BioFire® Respiratory Panel 2.1 among 17 pediatric patients in 2023.

Patient no. [a]	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Age, years	2	3	3	4	4	4	5	5	5	6	9	2	4	9	4	6	3
Coronavirus NL63						✓					✓			✓			✓
Coronavirus OC43			✓											✓			
SARS-CoV-2	✓			✓	✓	✓	✓		✓		✓		✓	✓	✓	✓	
Influenza virus A	✓	✓			✓	✓	✓	✓	✓	✓		✓	✓		✓	✓	✓
Influenza virus A/H1													✓				
Influenza virus A/H1-2009									✓				✓		✓	✓	✓
Influenza virus A/H3	✓	✓			✓	✓	✓	✓		✓			✓		✓	✓	
Influenza virus B																	
Parainfluenza virus 1					✓			✓		✓		✓	✓		✓		✓
Parainfluenza virus 2	✓	✓												✓		✓	✓
Parainfluenza virus 3			✓	✓						✓							
Parainfluenza virus 4			✓	✓						✓		✓		✓			✓
Adenovirus	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓			✓	✓	✓
Human metapneumovirus				✓			✓				✓	✓	✓				✓
Respiratory syncytial virus		✓	✓	✓	✓	✓		✓	✓			✓		✓	✓	✓	✓
Human rhinovirus/enterovirus	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Mycoplasma pneumoniae											✓						

[a] Patients with ≥6 pathogens detected were labeled from A to Q.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 3 Distribution and proportions of respiratory pathogens detected by the BioFire® Respiratory Panel 2.1, stratified by different age groups.

Pathogens	No. (%) of patients									
	<1 (n = 533)	1-2 (n = 819)	2-4 (n = 1435)	5-9 (n = 665)	10–17 (n = 383)	18-24 (n = 265)	25-34 (n = 486)	35-54 (n = 1089)	55-74 (n = 1483)	≥75 (n = 792)
Viruses										
Coronavirus 229E	0 (0.0)	1 (0.1)	3 (0.2)	3 (0.5)	2 (0.5)	2 (0.8)	2 (0.4)	5 (0.5)	0 (0.0)	1 (0.1)
Coronavirus HKU1	0 (0.0)	3 (0.4)	7 (0.5)	3 (0.5)	1 (0.3)	1 (0.4)	3 (0.6)	6 (0.6)	16 (1.1)	5 (0.7)
Coronavirus NL63	2 (0.4)	5 (0.6)	14 (1.0)	7 (1.1)	6 (1.6)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)
Coronavirus OC43	1 (0.2)	4 (0.5)	30 (2.1)	12 (1.8)	2 (0.5)	5 (1.9)	3 (0.6)	2 (0.2)	1 (0.1)	0 (0.0)
SARS-CoV-2	23 (4.3)	66 (8.1)	86 (6.0)	68 (10.2)	23 (6.0)	16 (6.0)	33 (6.8)	50 (4.6)	79 (5.3)	40 (5.5)
Influenza virus A	11 (2.1)	15 (1.8)	41 (2.9)	53 (8.0)	26 (6.8)	12 (4.5)	11 (2.3)	20 (1.8)	16 (1.1)	6 (0.8)
Influenza virus A/H1	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Influenza virus A/H1-2009	3 (0.6)	3 (0.4)	14 (1.0)	18 (2.7)	5 (1.3)	2 (0.8)	9 (1.9)	8 (0.7)	13 (0.9)	3 (0.4)
Influenza virus A/H3	2 (0.4)	6 (0.7)	23 (1.6)	41 (6.2)	20 (5.2)	10 (3.8)	6 (1.2)	13 (1.2)	6 (0.4)	2 (0.3)
Influenza virus B	0 (0.0)	1 (0.1)	2 (0.1)	1 (0.2)	1 (0.3)	0 (0.0)	1 (0.2)	2 (0.2)	3 (0.2)	0 (0.0)
Parainfluenza virus 1	6 (1.1)	17 (2.1)	77 (5.4)	53 (8.0)	3 (0.8)	0 (0.0)	2 (0.4)	1 (0.1)	1 (0.1)	0 (0.0)
Parainfluenza virus 2	0 (0.0)	6 (0.7)	11 (0.8)	21 (3.2)	3 (0.8)	0 (0.0)	2 (0.4)	2 (0.2)	2 (0.1)	0 (0.0)
Parainfluenza virus 3	45 (8.4)	118 (14.4)	212 (14.8)	42 (6.3)	14 (3.7)	3 (1.1)	4 (0.8)	7 (0.6)	11 (0.7)	5 (0.7)
Parainfluenza virus 4	4 (0.8)	23 (2.8)	70 (4.9)	39 (5.9)	2 (0.5)	1 (0.4)	1 (0.2)	4 (0.4)	5 (0.3)	2 (0.3)
Adenovirus	40 (7.5)	130 (15.9)	224 (15.6)	95 (14.3)	22 (5.7)	9 (3.4)	15 (3.1)	12 (1.1)	17 (1.1)	10 (1.4)
Human metapneumovirus	4 (0.8)	41 (5.0)	138 (9.6)	60 (9.0)	8 (2.1)	1 (0.4)	6 (1.2)	10 (0.9)	13 (0.9)	9 (1.2)
Respiratory syncytial virus	77 (14.4)	160 (19.5)	285 (19.9)	60 (9.0)	17 (4.4)	3 (1.1)	3 (0.6)	4 (0.4)	17 (1.1)	7 (1.0)
Human rhinovirus/ enterovirus	78 (14.6)	321 (39.2)	636 (44.3)	248 (37.3)	56 (14.6)	20 (7.5)	34 (7.0)	62 (5.7)	42 (2.8)	11 (1.5)
Bacteria										
Bordetella parapertussis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)
Bordetella pertussis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Chlamydophila pneumoniae	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycoplasma pneumoniae	0 (0.0)	0 (0.0)	2 (0.1)	6 (0.9)	4 (1.0)	0 (0.0)	1 (0.2)	4 (0.4)	1 (0.1)	0 (0.0)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

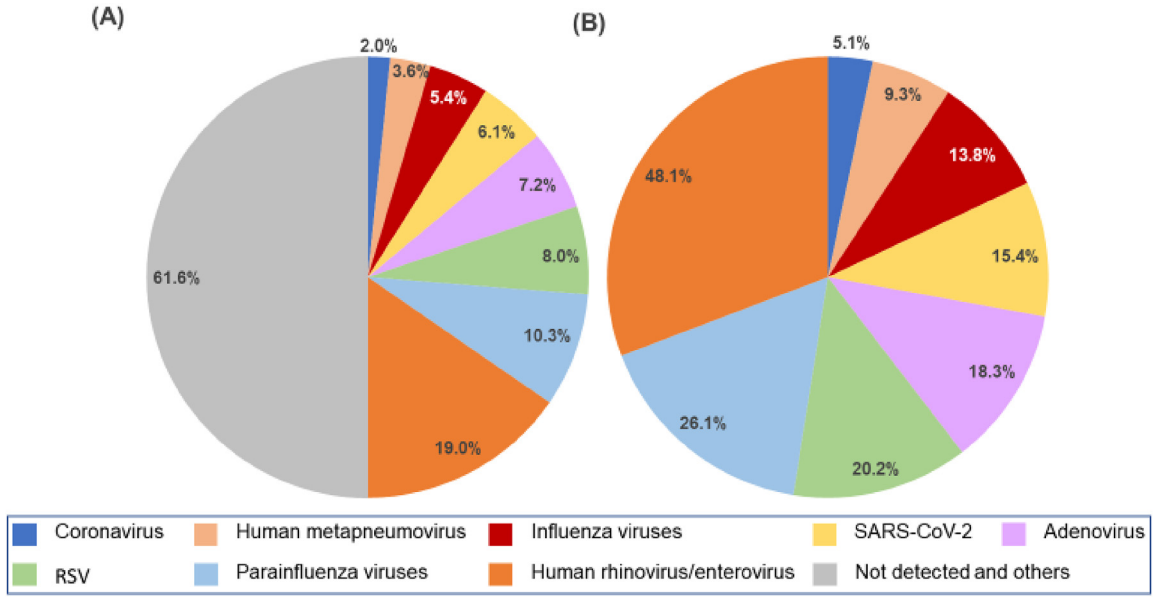


Figure 1. Viral pathogens detected by the BioFire® Respiratory Panel 2.1. (A) Of 7950 samples tested, 3133 (39.4%) were positive for at least one pathogen. (B) Among the samples with at least a pathogen detected, the distribution of viral pathogens is shown. Note: Coronaviruses include coronavirus 229E, HKU1, NL63, and OC43. Influenza viruses include influenza virus A, A/H1, A/H1-2009, A/H3, and B. Parainfluenza viruses include 4 human parainfluenza virus, type 1–4. The category of "Not detected and others" refers to negative samples or the detection of bacterial pathogens, including *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

Dynamic trends of respiratory pathogens and policies during COVID-19

We also considered the policies of Taiwan government and the course of the COVID-19 pandemic. The diversity of respiratory pathogen infections has increased over time (Fig. 3), for both pediatric and adult populations. In the pediatric group, cases of SARS-CoV-2 and influenza began to emerge in 2022, and the annual cases of human rhinovirus/enterovirus (n = 123 in 2020; 257 in 2021; 448 in 2022; 511 in 2023) showed an increasing trend. In adults, the cases of SARS-CoV-2 and influenza increased in 2022 compared to 2020 and 2021. The number of influenza cases (n = 278) significantly elevated in 2023 compared to SARS-CoV-2 cases (n = 149). Moreover, adult RSV cases exhibited an upward trend, with 2 cases in 2020, 3 in 2021, 8 in 2022, and 21 in 2023.

RSV incidence and policy impact

In response to vaccine policies, we also analyzed the incidence rate of annual RSV cases among three groups recommended for vaccination (Table 4). The incidence among the elderly group (aged 60 years and older) has shown a steady increase from 2020 to 2023, with rates of 0%, 0.4%, 0.7%, and 0.8%, respectively. Fig. 4 also briefly summarizing, the non-pharmaceutical interventions (NPI) and quarantine measures implemented by the Taiwan government began in January 2020 and were relaxed in May 2022.[12] It can be observed that the cases of RSV between January 2020 and May 2022 were fewer compared to those after May 2022, concentrating particularly between September and December.

Discussion

We analyzed the onset of COVID-19 until the end of 2023, encompassing the Taiwan government's implementation of isolation policies from the beginning of COVID-19 pandemic. In response to the pandemic, Taiwan adopted various infection control measures, such as universal mask-wearing, increased face mask production, hand hygiene practices, border control, digital technology use, quarantine, and travel restrictions.[12] Recurrent pandemics arising from respiratory pathogens constitute a significant global health menace, given their unpredictable nature and potential to inflict profound harm on health, societies, and economies. The ongoing COVID-19 pandemic serves as a catalyst for a renewed focus on pandemic preparedness, drawing on the challenges confronted and lessons discerned throughout this crisis. In 2023, the World Health Organization (WHO) introduced the Preparedness and Resilience for Emerging Threats (PRET) initiative, coinciding with the transition of most health systems from an acute pandemic response mode to the sustained, longer-term management of COVID-19, alongside other respiratory pathogens.[13]

Virus groups with epidemic and pandemic potential, such as adenovirus, coronavirus, human rhinovirus/enterovirus, influenza, RSV, human metapneumovirus, and parainfluenza, have exhibited notable changes in respiratory virus infections worldwide during the COVID-19 pandemic, with variations observed among different virus types.[14] As Table 3 illustrates, variations in the pathogens contracted among different age groups were observed in the subgroup analysis, highlighting differences in prevalent infections at the onset and relaxing of COVID-19 pandemic isolation policies. During this shift, PRET also actively

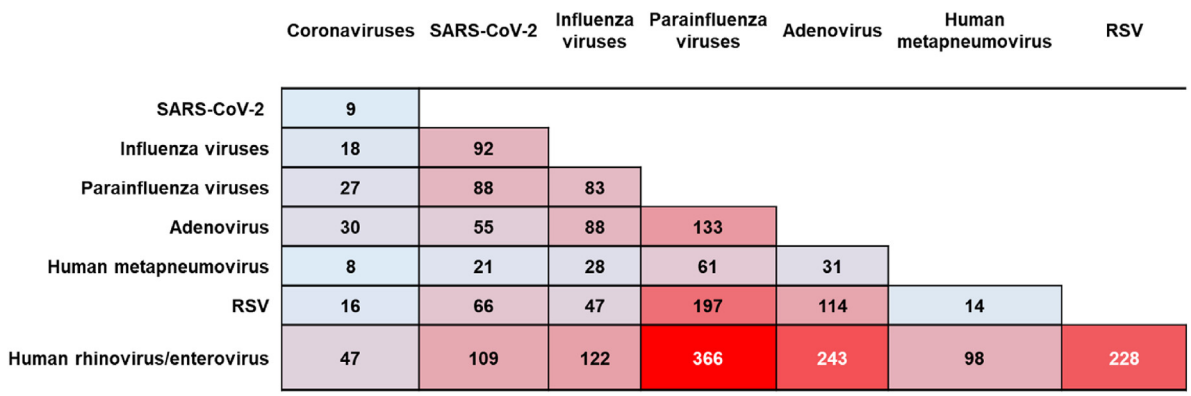
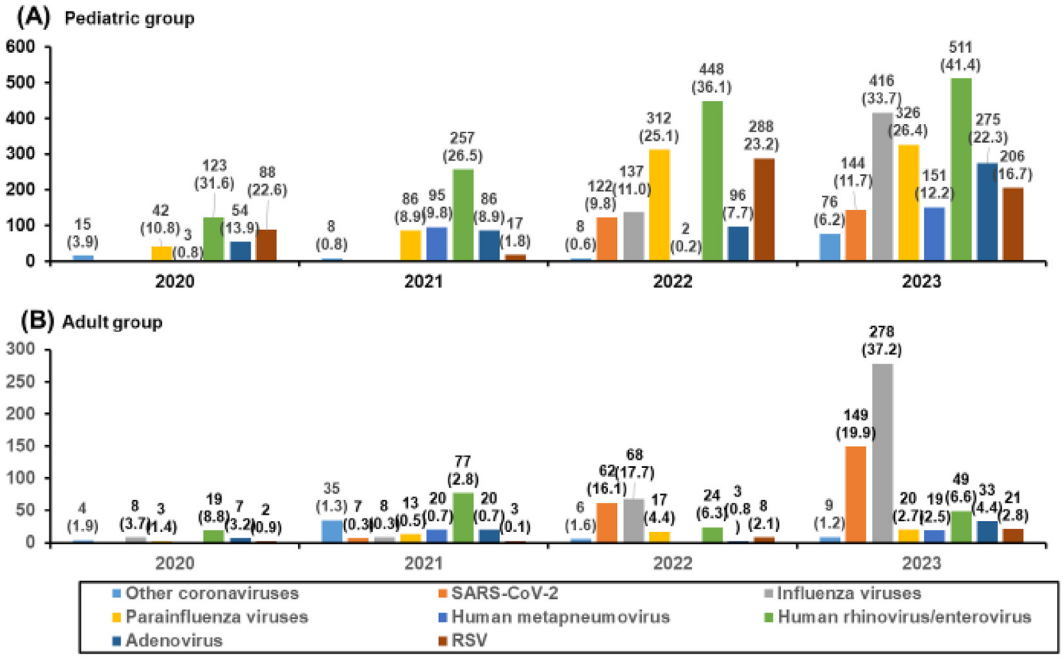


Figure 2. Frequency of the detection of multiple viral pathogens by the BioFire® Respiratory Panel 2.1. The numbers in the boxes represent the count of samples with mixed detection of two specific pathogens in each positive sample. Human rhinovirus/enterovirus was most commonly detected in conjunction with parainfluenza viruses, followed by adenovirus and respiratory syncytial virus (RSV).
Note: Coronaviruses include coronavirus 229E, HKU1, NL63, and OC43. Influenza viruses include influenza virus A, A/H1, A/H1-2009, A/H3, and B. Parainfluenza viruses include human parainfluenza virus type 1—4.

engaged in identifying both strengths and gaps in response to the COVID-19 pandemic and underscored the importance of local capabilities or timely access to genomic sequencing for pathogens with pandemic and epidemic potential. The routine implementation of molecular point-of-care tests not only reduced the time to obtain results and the duration of stay in admission cohort areas but also sustained molecular and genomic sequencing capacities established for SARS-CoV-2 at national and sub-national levels, facilitating respiratory pathogen monitoring.[15] Emphasizing the importance of local capability for timely genomic sequencing of pathogens with pandemic and epidemic potential, in a hospital setting, multiplex PCR appears to be more reliable than a rapid antigen test and better aligns with clinical needs.[13] Although the multiplex PCR testing is more sensitive and convenient, the cost and the availability should be taken into consideration when generalizing this test in clinical setting.

In a recent study conducted in Japan using the BioFire FilmArray Respiratory Panel 2.1, at least one virus was detected in 32 (16.8%) out of 191 patients. The most frequently identified were human rhinovirus/enterovirus (5.8%, n = 11), human metapneumovirus (3.7%, n = 7), coronavirus 229E (2.1%, n = 4), and coronavirus OC43 (1.6%, n = 3), while no influenza viruses were detected. SARS-CoV-2 was found in 4.2% of patients (n = 8) who were negative for other respiratory viruses. The mixed detection rate was 1.0%, with adenovirus co-infections with either human rhinovirus/enterovirus or human metapneumovirus. The study was conducted at the beginning of the COVID-19 pandemic, during aggressive non-pharmaceutical interventions (NPI).[16] However, prior to the COVID-19 era, another Japanese study using multiplex PCR found a virus-bacteria co-infection rate of 2.8% (3/108) in adults.[17] As revealed in our study, a higher mixed detection rate in children, increasing after the COVID-19 lockdown was lifted, suggesting that age groups influence mixed detection rates. Considering the viral pathogen infection patterns in different age groups, a follow-up Japanese study during strict quarantine found an 80.2% negative rate using multiplex PCR. Age distribution was 0—19 years (3.0%, n = 10), 20—39 years (22.6%, n = 74), 40—59 years (22.6%, n = 74), 60—79 years (30.5%, n = 100), and over 80 years old (21.3%, n = 70). The positivity rate was only 3% among children,[8] differing from our findings of higher detection rates in the pediatric group. This discrepancy may be due to variations in study periods and patient populations. Our longer study period observed respiratory infection patterns before and after NPI implementation.

RSV significantly contributes to the global morbidity and mortality burden in children, being a leading cause of acute respiratory tract infections in young children and associated with higher in-hospital death rates than in the community.[18] Exploring passive immunization for targeted protection against RSV could potentially reduce the disease burden associated with it.[18] Moreover, according to previous studies, there is also a high incidence among older hospitalized adults and vulnerable adults before the COVID-19 pandemic.[19] Before COVID-19, RSV epidemics usually followed a biennial oscillation pattern, peaking in winter in temperate northern regions and during rainy seasons in tropical and subtropical areas.[20,21] Following the emergence of SARS-CoV-2 and the onset of the COVID-19 era, there was a significant reduction in RSV activity.[12] In Taiwan, mirroring this trend, the traditional RSV peak season in spring and fall experienced a shift to winter in 2020, attributed to the implementation of COVID-19 measures.[22] Similarly, in our study, we observed pattern of seasonal RSV cases shifted to September and December. Since humans are the only known reservoir of RSV, viral transmission is largely influenced by seasonal changes associated with environmental and human behavioral factors like travelling, indoor gathering, population density, temperature and humidity.[23] As depicted in Table 4, The RSV incidence in the pediatric group shows a decreasing trend. During the COVID-19 pandemic, despite a very low number of cases in adults, the incidence rate exhibited a significant increase pattern after the relaxation of isolation policies in Taiwan. Globally, in the initial year of the COVID-19 pandemic, shortly after the aggressive implementation



Note: RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Other coronaviruses include coronavirus 229E, HKU1, NL63, and OC43. Influenza viruses include influenza virus A, A/H1, A/H1-2009, A/H3, and B. Parainfluenza viruses include human parainfluenza virus type 1-4.

Figure 3. Annual distribution of viral pathogens detected by the BioFire® Respiratory Panel 2.1 in the pediatric (aged less than 18 years, A) and adult (aged 18 years or older, B) group from January 2020 to November 2023. The number in the brackets represents the detection rate of the individual virus. The detection rate is defined as the number of positive samples for the pathogen in that population for the year divided by the total number of individuals tested in that population for the same year.
Note: RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Other coronaviruses include coronavirus 229E, HKU1, NL63, and OC43. Influenza viruses include influenza virus A, A/H1, A/H1-2009, A/H3, and B. Parainfluenza viruses include human parainfluenza virus type 1—4.

of NPI around February—March 2020, RSV cases immediately dropped worldwide. As these measures were gradually eased, various regions across the globe witnessed varying degrees of off-season resurgence of cases,[12] consistent with our study. In Taiwan, the Central Epidemic Command Center (CECC) made policy adjustments in May 2022, announcing the relaxation of isolation measures, with individuals who had received the COVID-19 vaccine being eligible for exemption from isolation. Subsequently, in July 2022, Taiwan witnessed the emergence of the Omicron BA.5 variant, marking the onset of the community outbreak of COVID-19.[24] This may explain the off-season resurgence of RSV cases after May 2022 and subsequently demonstrating the typical seasonal pattern.

Simultaneously, within the realm of community protection, there is a strong emphasis on the crucial role of vaccination in controlling and mitigating the impact of pandemics.[13] The introduction of COVID-19 vaccines marked the beginning of vaccine policy development.[24,25,26] Subsequently, the COVID-19 vaccine has proven effective in preventing symptomatic illness, lowering the likelihood of hospitalization, and reducing mortality risks.[27] An unexpected surge in pneumococcus vaccination occurred in Taiwan, driven by increased demand due to the COVID-19 pandemic and also resulting in shipping restrictions in Japan.[28,29]

The pros of this study include a large clinical sample size, a significant number of representative cases, and extensive pathogen investigation using the BioFire Respiratory Panel. However, this study still has some limitations. The major limitation is the absence of clinical symptoms and disease data. This information is essential for interpreting the relevance of the detected respiratory organisms. Without clinical context, it is challenging to determine whether an organism is merely part of the normal flora or a true pathogen causing infection. The correlation between clinical diseases and detected pathogens is valuable in epidemiological and clinical information, and its absence limits the ability to draw comprehensive conclusions about the pathogenic significance of the detected organisms. Nevertheless, the aim of this study is to demonstrate that multiplex PCR can offer rapid pathogen identification, providing clinicians with valuable clues for making rapid and convenient diagnoses in clinical

Table 4 Annual incidence rates[a] of respiratory syncytial virus (RSV), stratified by age from January 2020 to November 2023.

Year (no. of samples with a positive result)	No. (%) of patients				
	Infant (age ≤2 years)	Young children (age >2 - ≤5 years)	School children (age >6-≤17 years)	Adult (age ≥18-≤59 years)	Elderly (age ≥60 years)
2020 (n = 290)	58 (20.0)	24 (8.3)	6 (2.1)	2 (0.7)	0 (0.0)
2021 (n = 564)	6 (1.1)	10 (1.8)	1 (0.2)	1 (0.2)	2 (0.4)
2022 (n = 947)	181 (19.1)	88 (9.3)	19 (2.0)	1 (0.1)	7 (0.7)
2023 (n = 1332)	101 (7.6)	77 (5.8)	28 (2.1)	10 (0.8)	11 (0.8)

[a] The number of patients with RSV detected by the BioFire® Respiratory Panel 2.1, divided by the total number of patients with positive samples that year.

settings. Another limitation is the absence of validation for mixed detection or codetection using BioFire RP 2.1, despite its previously demonstrated excellent performance.[5] Additionally, the FilmArray panel did not include SARS-CoV-2 at the beginning of Taiwan's endemic; it was only incorporated after July 1, 2021. In a recent study, the in-house multiplex PCR assay exhibited high sensitivity (95.0%), specificity (93.8%), positive predictive value (PPV) of 99.0%, and a negative predictive value (NPV) of 75.0%.[30] Out of the 1612 specimens tested with the BioFire Respiratory panel, at least one pathogen was detected in 1,020, yielding an overall positivity rate of 63.3%. The panel consistently demonstrated a positive percent agreement of 91.7% or greater and a negative percent agreement of 93.8%.[5] Indeed, viral co-detections for acute respiratory illnesses are more common in children than in adults.[31] As our findings also show simultaneous detection of multiple pathogens. Previous research recorded mixed detection or codetection, revealing that 21% (20/95) of patients were dual-positive, 4% (4/95) were triple-positive for respiratory pathogens, resulting in an overall co-detection rate of 25% (24/95).[30] Notably, SARS-CoV-2 was frequently mixed-detected with adenovirus and human rhinovirus/enterovirus.[30] In another study comparing FilmArray Respiratory Panel (FilmArray RP) with in-house multiplex PCR, the dual viral detection rate was found to be 16.2%, with a primary

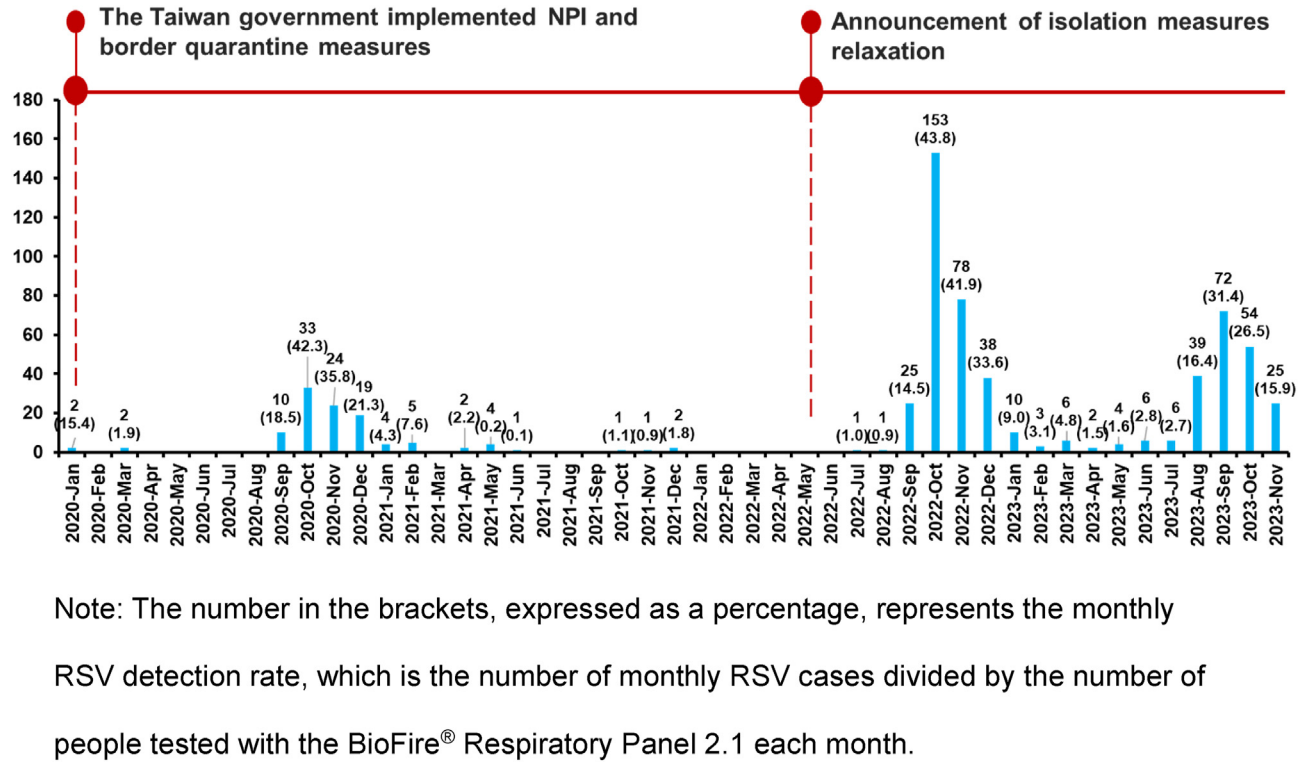


Figure 4. Monthly numbers of all samples with respiratory syncytial virus detected by the BioFire® Respiratory Panel 2.1 from January 2020 to November 2023. The timing of the initiation and relaxation of Taiwan's government COVID-19 policies is highlighted. Non-pharmaceutical interventions (NPIs) include the three-tier safety stockpiling framework for personal protective equipment, the release of reserve masks, restrictions on the export of medical masks, and government-distributed masks for sale. Note: The number in the brackets, expressed as a percentage, represents the monthly RSV detection rate, which is the number of monthly RSV cases divided by the number of people tested with the BioFire® Respiratory Panel 2.1 each month.

association with RSV combined with other respiratory viruses.[34] Another study investigating 160 samples by using BioFire RP2.1, where RSV predominated, and human rhinovirus/enterovirus followed, the dual-detection rate was 23.8% (19/80) and 24.3% (20/80), whether SARS-CoV-2 was positive or not. Among SARS-CoV-2 positive samples, other pathogens were identified in almost 24% on BioFire RP2.1 and in 11.8% on another commercial multiplex PCR assay.[32] The diagnostic accuracy of BioFire RP2.1 cannot be substantiated, given the absence of comparison tests, lack of standardization of assays, and the non-validation of the multiplex PCR assay.[33] Factors contributing to these differences include variations in sample size, sample type, timing of sample collection, differences in collection, methodology, cross-reactivity[5] and viral species with sensitivities of 80% for parainfluenza viruses and 83.3% for RSV, compared to multiple PCR.[34] This limitation warrants further study to integrate corresponding conventional methods, such as observing cytopathic effects in cell culture, direct fluorescent antibody assay, immunochromatographic antigen testing, serological methods, rapid antigen tests, next generation sequencing, as well as comparative multiplex PCR to validate the true co-detection rate, especially in the pediatric group. The last is that there are still too few cases of adult RSV, but there is a growing trend. Perhaps it can correspond to global vaccine policies.

In conclusion, the diversity of upper respiratory infection pathogens in children highlights the potential of multiplex PCR for rapid and convenient pathogen identification, despite concerns about accuracy with increasing mixed detection rates. This study underscores respiratory pathogen dynamics and the role of multiplex PCR in diagnosis, offering insights for age-specific vaccine policy considerations in response to the evolving landscape of respiratory infections.

Ethics approval

The institutional review board of China Medical University Hospital waived the requirement for written informed consent because the study involved minimal risk to the patients (IRB number: CMUH112-REC3-041).

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CRediT authorship contribution statement

Hung-Chieh Su: Writing — review & editing, Writing — original draft. **Yu-Chang Chang:** Visualization, Validation. **Chih-Hao Chen:** Writing — review & editing, Supervision, Software. **Meng-Yu Cheng:** Data curation, Conceptualization. **Wen-Hsin Hsih:** Funding acquisition, Formal analysis. **Yi-Jhen Chen:** Methodology, Investigation. **Chia-Huei Chou:** Project administration, Methodology. **Yu-Chao Lin:** Software, Resources. **Chiung-Tzu Hsiao:** Resources, Project administration. **Hong-Mo Shih:** Validation, Supervision. **Mao-Wang Ho:** Writing — review & editing, Project administration, Data curation. **Po-Ren Hsueh:** Writing — review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interests

All authors have no conflicts of interest to declare.

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