OPEN

Respiratory Viral Co-infection in SARS-CoV-2-Infected Children During the Early and Late Pandemic Periods

Jianling Xie, MD, MPH, Todd A. Florin, MD, MSCE,† Anna L. Funk, PhD, MSc,* Daniel J. Tancredi, PhD,‡ Nathan Kuppermann, MD MPH,§ and Stephen B. Freedman MDCM, MSc¶ on behalf of the Pediatric Emergency Research Network (PERN)-COVID-19 and Pediatric Emergency Research Canada (PERC) Study Teams*

Background: Knowledge regarding the impact of respiratory pathogen co-infection in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected children seeking emergency department care is limited, specifically as it relates to the association between SARS-CoV-2 viral co-infection and disease severity and factors associated with co-infection.

Methods: This secondary analysis included data from 2 prospective cohort studies conducted between March 2020 and February 2022 that included children <18 years of age tested for SARS-CoV-2 infection along with additional respiratory viruses in a participating emergency department. Outcomes included the detection rate of other respiratory viruses and the occurrence of severe outcomes (ie, intensive interventions, severe organ impairment and death).

Results: We included 2520 participants, of whom 388 (15.4%) were SARS-CoV-2-positive. Detection of additional respiratory viruses occurred in 18.3% (71/388) of SARS-CoV-2-positive children, with rhinovirus/enterovirus being most frequently detected (42/388; 10.8%). In multivariable analyses (adjusted odds ratio and 95% confidence interval), among SARS-CoV-2-positive children, detection of another respiratory virus was not associated with severe outcomes [1.74 (0.80–3.79)], but detection of rhinovirus/enterovirus [vs. isolated SARS-CoV-2 detection 3.56 (1.49–8.51)] and having any preexisting chronic medical condition [2.15 (1.06–4.36)] were associated with severe outcomes. Among SARS-CoV-2-positive children, characteristics independently associated with an increased odds of any other viral co-infection included: age and delta variant infection.

Conclusions: Approximately 1 in 5 children infected with SARS-CoV-2 had co-infection with another respiratory virus, and co-infection with rhinovirus/enterovirus was associated with severe outcomes. When public health restrictions were relaxed, co-infections increased.

Key Words: child, respiratory viral co-infection, severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, severe outcome

(*Pediatr Infect Dis J* 2025;44:333–341)

As of August 2024, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus continues to circulate, and as we prepare for the annual winter surge in pediatric viral illness, there remains a need to understand the impact of respiratory virus co-infection in SARS-CoV-2 infected individuals.[1] This is a particularly important pediatric concern as children are more likely to have respiratory viral co-infection.[2,3] Pre-coronavirus disease 2019 (COVID-19) pandemic data are inconsistent about whether viral respiratory pathogen co-infection is associated with increased disease severity.[4] A meta-analysis assessing clinical disease severity among hospitalized individuals comparing detection of single versus multiple viruses was hampered by the presence of significant heterogeneity across almost all outcomes.[3] However, hospitalized children with bronchiolitis and viral co-infections had reduced lengths of stay,[3] while a single study reported higher mortality in Cambodian preschool children with viral co-infections.[5] More recently, the co-infection of respiratory syncytial virus (RSV) and rhinovirus/enterovirus has been associated with increased illness severity among children <5 years of age hospitalized with SARS-CoV-2.[6]

The reported prevalence of co-infection in SARS-CoV-2-infected individuals is inconsistent. Although a study from 2020 reported that among a predominantly adult population, evaluated in clinics, EDs and inpatient wards, the co-infection rate was 21%,[7] a study completed in 2022 reported that co-infections occurred in only 5% of symptomatic school-age, SARS-CoV-2-infected children who participated in a community surveillance study.[8] In

Accepted for publication October 1, 2024

From the *Department of Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada; †Department of Pediatrics, Feinberg School of Medicine, Northwestern University and Division of Emergency Medicine, Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois; ‡Department of Pediatrics, University of California, Davis, California; §Children's National Hospital, Washington, DC; and ¶Sections of Pediatric Emergency Medicine and Gastroenterology, Departments of Pediatrics and Emergency Medicine, Cumming School of Medicine, University of Calgary, Calgary, Canada.

Supported by grants from the Public Health Agency of Canada's Immunization Partnership Fund (Emerging Issues Fund) and their Emerging Issues Fund (Enhanced Surveillance), and the Canadian Institute of Health Research (Operating Grant: Emerging COVID-19 Research Gaps and Priorities Funding Opportunity and Operating Grant: COVID-19—Clinical Management), Alberta Innovates, the Alberta Health Services—University of Calgary—Clinical Research Fund, the Alberta Children's Hospital Research Institute, the COVID-19 Research Accelerator Funding Track (CRAFT) Program at the University of California, Davis, and the Cincinnati Children's Hospital Medical Center Division of Emergency Medicine Small Grants Program. A.L.F. was supported by the University of Calgary Eyes-High Post-Doctoral Research Fund. N.K. was supported by the Bo Tomas Brofeldt Endowed Chair at the University of California, Davis. S.B.F. was supported by the Alberta Children's Hospital Foundation Professorship in Child Health and Wellness.

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada.

S.B.F. had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Data will be shared, upon reasonable request, for academic purposes, with appropriate individuals who have obtained appropriate ethics permissions and data sharing agreements.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).

Address for correspondence: Stephen B. Freedman, MDCM, MSc, Departments of Pediatrics and Emergency Medicine, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, C4-634 28 Oki Drive NW, Calgary, Alberta T3B 6A8, Canada. E-mail: Stephen.freedman@ahs.ca.

ISSN: 0891-3668/25/444-333341
DOI: 10.1097/INF.0000000000004623

contrast to the latter study, in a large US cohort of hospitalized SARS-CoV-2-infected children evaluated between March 2020 and February 2022, 21% had respiratory viral co-infection.[6] In this study, co-infected children were more likely to be <5 years old, receive oxygen support and be admitted to the intensive care unit.[6] Although other studies have evaluated this association among hospitalized children,[6] and in a mixed hospital-based population [ie, emergency department (ED), intensive care unit, inpatient, outpatient and operating room],[1] none were restricted to children seeking ED care.

To better understand the impact of respiratory viral co-infection in SARS-CoV-2-infected children seeking ED care, we conducted a secondary analysis of a multinational database that combined two prospective cohort studies. We sought to determine the proportion of SARS-CoV-2-positive participants with respiratory viral co-infection and if co-infection is associated with the development of severe disease. Additionally, we sought to identify factors associated with SARS-CoV-2 viral co-infection.

MATERIALS AND METHODS

Design and Setting

This study combined data from 2 prospective cohort studies: the (1) Pediatric Emergency Research Network[9] (PERN)-COVID-19 study that recruited participants in 41 pediatric EDs between March 7, 2020 and June 15, 2021 and (2) Pediatric Emergency Research Canada (PERC)[10] COVID-19 study that recruited participants in 14 Canadian pediatric EDs between August 4, 2020, and February 22, 2022. In the PERN study, eligible participants were enrolled in EDs in 10 countries (Argentina, Australia, Canada, Costa Rica, Italy, New Zealand, Paraguay, Singapore, Spain and the United States).[11] In both studies, participating sites obtained local ethics review board approval or established a reliance agreement with the Cincinnati Children's Hospital Medical Center institutional review board. The legal guardians of all participants provided informed consent, and children provided assent, as appropriate.

Participants and Recruitment

In the parent study, eligible study participants were <18 years old and had specimens obtained for SARS-CoV-2 nucleic acid testing because of symptoms or epidemiologic risk factors. In this secondary analysis, we required patients to have a respiratory multiplex panel test performed. Children were eligible for study participation regardless of the result of SARS-CoV-2 testing. Exclusion criteria included: (1) duplicate enrollment (ie, enrolled in both the PERN and PERC-COVID-19 studies as some institutions contributed data to both studies); (2) declined permission for secondary use of data; (3) no SARS-CoV-2 test result available and (4) absence of symptoms potentially associated with SARS-CoV-2 infection or known exposure. Recruitment aimed for consecutive enrollment, but modifications were required to balance test-positive and negative participants as has been described.[11,12] Testing indications varied by institution and country and were modified as the pandemic evolved.

Objectives and Outcomes

We sought to determine the overall and individual virus co-infection rates of SARS-CoV-2 along with other respiratory viruses and to determine if viral co-infection is associated with severe outcomes. We also sought to determine if the detection of a non-SARS-CoV-2 virus was associated with SARS-CoV-2 test result status (ie, positive or negative) and to identify factors associated with viral co-infection among SARS-CoV-2-positive participants.

Data Collection

The PERN and PERC studies implemented harmonized recruitment procedures and data collection tools. We collected participant demographic, epidemiological and clinical data during the index ED visit or shortly thereafter via interviews with participants' caregivers. Information regarding testing and interventions performed during the index ED visit and subsequent hospitalization, if required, was extracted from the medical record. We standardized study procedures using a manual of operations. To classify outcomes following the ED visit we completed a medical record review and follow-up telephone or email/text survey 14 days following the index ED visit. Participants were considered lost to follow-up if 5 telephone follow-up attempts were unsuccessful, even if their medical records were reviewed.

Definitions and Microbiologic Diagnostics

SARS-CoV-2 status was classified as "positive" if a nucleic acid test performed on a swab from the nares, nasopharynx, or oral cavity was positive at the index ED visit or during the subsequent 14 days. As this was an observational study, we did not specify the approach to SARS-CoV-2 detection. Moreover, local SARS-CoV-2 specimen acquisition and testing procedures evolved over time based on access to swabs, reagents, regional epidemiology and scientific advances. In accordance with accepted standards for defining hospital-acquired infections,[13] we defined co-infection as having a positive testing result on a multiplex viral panel in a respiratory sample collected at or within 2 days following the index ED visit. Positive tests collected more than 2 days after the index ED visit were considered to be secondary infections (ie, not co-infections). We collected data regarding nucleic acid test results for influenza A, influenza B, RSV and any other respiratory viruses included in multiplex viral panels. As multiplex specimen testing was part of local clinical care, testing was performed using the panels selected by local laboratories, and therefore viral targets varied by site.

We defined a severe outcome as the occurrence of any of the following events within 14 days after the index ED visit[12]: cardiac or cardiovascular (eg, cardiac arrest, cardiac ischemia, congestive heart failure, endocarditis, myocarditis, pericarditis or stroke), infectious (eg, disseminated intravascular coagulation, mastoiditis, sepsis with bacteremia, septic shock or toxic shock syndrome), neurologic (eg, encephalitis and meningitis), respiratory (eg, acute respiratory distress syndrome, empyema, necrotizing or cryptogenic organizing pneumonia, pleural effusion or pneumothorax or pneumomediastinum requiring drainage or respiratory failure) and death. Performance of any of the following interventions was also deemed to represent a severe outcome: chest drainage, extracorporeal membrane oxygenation, high flow oxygen by nasal cannula, inotropic support, positive pressure ventilation and renal replacement therapy. The diagnosis of multisystem inflammatory syndrome in children or Kawasaki disease was reported as assigned by the clinical care teams and was considered severe if accompanied by one of the diagnoses or interventions.[12]

Sample Size

This was a secondary analysis of the parent PERN and PERC-COVID-19 prospective cohort studies.[11,14] The sample size specification for the PERN-COVID-19 parent study included the recruitment of up to 12,500 participants to acquire 50+ COVID-positive children who experienced severe outcomes, the parent study's primary objective.[11] When the parent study achieved the desired number of children, recruitment was terminated. For the PERC-COVID-19 study, data were shared in real-time with the Public Health Agency of Canada for pandemic surveillance purposes and recruitment was terminated after the funding period.

As such, no formal sample size calculations were performed for the current analysis.

Statistical Analysis

Viral co-infection rates were reported using descriptive statistics. χ^2 or Fisher exact tests and Mann-Whitney *U* tests were used for between-group univariate analyses comparing participants with to those without co-infection. Among SARS-CoV-2-infected children, we performed a multiple logistic regression analysis utilizing a model fitted with a generalized estimating equation accounting for study site, to assess if, overall, any viral co-infection is associated with severe outcomes. The following covariates were included in the model: viral co-infection (yes vs. no), age (continuous) and preexisting chronic medical condition (yes vs. no). We further evaluated the associations between severe outcomes and viral co-infection on an individual virus level using regression models with the same model specifications.

Among children with and without SARS-CoV-2-infected children, we assessed if SARS-CoV-2 detection was associated with the detection of other viruses, and performed a multivariable logistic regression model fitted with generalized estimating equation, including the following covariates: SARS-CoV-2 status indicator (positive vs. negative), age group, sex, baseline symptom duration, preexisting chronic medical condition, occurrence of any severe outcome and SARS-CoV-2 testing date (grouped in 3-month blocks). As the inclusion of sites that recruited small numbers of children could introduce bias by providing samples of participants that are not reflective of the presumed larger number of potentially eligible patients, we conducted a sensitivity analysis excluding those sites with fewer than 20 participants included in the regression models.

Study participants who did not have multiplex viral panels performed were excluded from the final analysis. Statistical significance was set at 0.05 for all tests. Analyses were 2-tailed and conducted using SPSS 25.0 (Armonk, NY: IBM Corp.).

RESULTS

Between March 2020 and February 2022, 18,019 children were enrolled, 2520 (14.0%) of whom were tested for SARS-CoV-2 and had respiratory multiplex panel performed within 2 days of the index ED visit and were not missing data on severe outcomes (Fig. 1). The median age was 2.0 years (IQR, 0.8–5.0), and 1375 (54.6%) were male. Fifteen percent (388/2520) of participants tested positive for SARS-CoV-2, of whom 242 (62.4%) were classified as infected with wild-type (Table 1). Among SARS-CoV-2-infected children, those included in this analysis compared with ineligible participants were younger, more likely to be hospitalized and to experience severe outcomes (see Table, Supplemental Digital Content 1, http://links.lww.com/INF/F813).

Viral Co-infection

Among 388 SARS-CoV-2-positive participants who were also tested for other viruses, 18.3% (71/388) of participants and 16.3% (31/190) of those hospitalized, had one or more additional viruses detected. Rhinovirus/enterovirus were the most frequently detected viruses among SARS-CoV-2-positive children (42/388; 10.8%) (see Table, Supplemental Digital Content 2, http://links.lww.com/INF/F814). Ten (2.6%) of the 388 SARS-CoV-2-positive children had 2 or more additional viruses detected. The co-infection rate was higher during the late phase of our study phase [March 2021–Feb 2022; 23.4% (41/175)], compared to the early [March 2020–Feb 2021; 14.1% (30/213)] study phase [difference 9.3%, 95% confidence interval (CI): of the difference 1.5–17.2] (see Figure, Supplemental Digital Content 3, http://links.lww.com/INF/F815). Among the 2132 SARS-CoV-2-negative children, 583 (27.3%) had at least 1 respiratory virus detected, with rhinovirus/enterovirus being the most frequently detected viruses (299/2132; 14.0%); 2.5% (54/2132) had more than 1 virus detected (Fig. 2; see Table, Supplemental Digital Content 2, http://links.lww.com/INF/F814).

Severe Outcomes

During the 14-day follow-up period, 11.1% (43/388) of 388 SARS-CoV-2-infected children experienced a severe outcome. In the unadjusted analyses among the SARS-CoV-2-positive children, we found no difference in the occurrence of severe outcomes, hospitalization, intensive care unit admission, health care revisits and death within 14 days between those with versus without co-infection (Table 2). In the multivariable analysis, the overall detection of viruses in addition to SARS-CoV-2 was not independently associated with the occurrence of a severe outcome [adjusted odds ratio (aOR): 1.74; 95% CI: 0.80–3.79). At an individual virus level, rhinovirus/enterovirus co-infection with SARS-CoV-2, compared to isolated SARS-CoV-2 detection, was associated with the occurrence of a severe outcome (aOR: 3.56; 95% CI: 1.49–8.51). In addition, older age and presence of a preexisting chronic medical condition were also associated with the occurrence of a severe outcome (Table 3). Among the SARS-CoV-2-negative study participants, 4.9% (26/529) who had single virus detection and 5.6% (2/36) of the children who had rhinovirus/enterovirus and other virus co-infection experienced a severe outcome.

Characteristics Associated with SARS-CoV-2 Viral Co-infection

Of the 71 children with SARS-CoV-2 viral co-infection, 55 (77%) were younger than 5 years. Among the 26 children with the SARS-CoV-2 delta variant, 10 (38%) had an additional virus detected (Table 2). In the multivariable analysis including all eligible children, SARS-CoV-2-positive children were less likely to have another virus detected (aOR: 0.51; 95% CI: 0.36–0.70); see Table, Supplemental Digital Content 4, http://links.lww.com/INF/F816. In the model including only SARS-CoV-2-positive children, age and infection with the delta variant were independently associated with any respiratory viral co-infection. In the model evaluating co-infection with rhinovirus/enterovirus, it was associated with having a severe outcome (aOR: 3.39; 95% CI: 1.42–8.10); see Table, Supplemental Digital Content 5, http://links.lww.com/INF/F817. Given the small number of participants with the co-infection of other viruses, individual virus regression analyses were not performed.

Sensitivity Analysis

In the sensitivity analysis, after excluding 105 SARS-CoV-2 test-negative and 66 test-positive participants from study sites with limited recruitment, the overall findings remained consistent, with the exception that the association between experiencing a severe outcome and detection of a non-SARS-CoV-2 respiratory virus was no longer statistically significant (aOR: 1.44; 95% CI: 0.92–2.25).

DISCUSSION

Among SARS-CoV-2-infected children tested for influenza A, influenza B, RSV, and any other respiratory viruses, 18% had an additional virus detected, with rhinovirus/enterovirus being the most common. Although, overall, respiratory virus co-infection was not associated with severe outcomes, viral co-infection along with SARS-CoV-2 was associated with severe outcomes among children 1–5 years old. SARS-CoV-2 viral co-infection was most common in young children and most frequently occurred during the later part

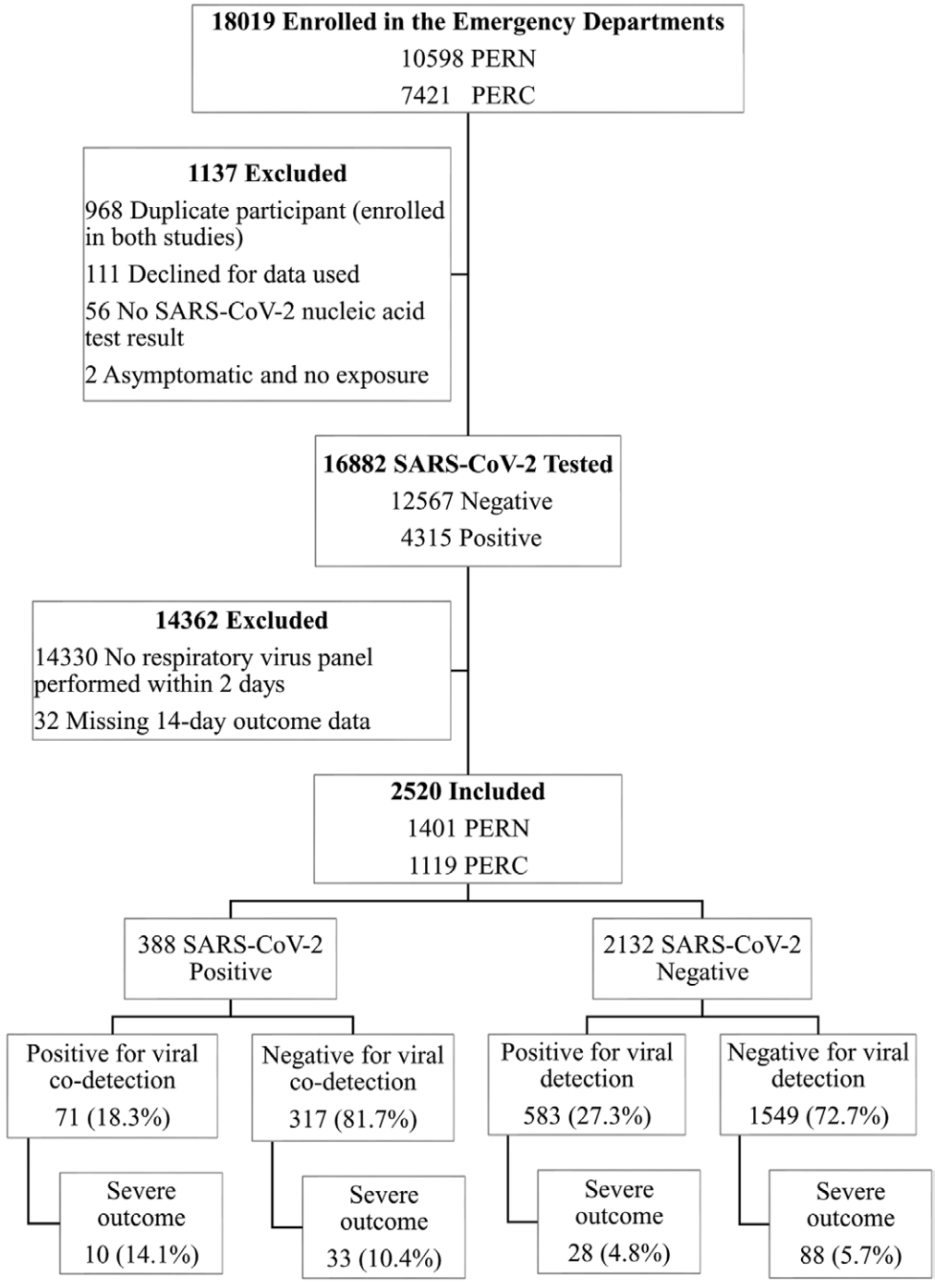

FIGURE 1. Study participants and outcomes*.*A severe outcome was defined based on the occurrence of any of the following events at or within 14 days of the index ED visit: cardiac or cardiovascular (eg, cardiac arrest, cardiac ischemia, congestive heart failure, endocarditis, myocarditis, pericarditis and stroke), infectious (eg, disseminated intravascular coagulation, mastoiditis, sepsis with bacteremia, septic shock and toxic shock syndrome), neurologic (eg, encephalitis and meningitis), respiratory (eg, acute respiratory distress syndrome, empyema, necrotizing or cryptogenic organizing pneumonia, pleural effusion or pneumothorax or pneumomediastinum requiring drainage and respiratory failure) and death. Performance of any of the following interventions was also deemed to represent a severe outcome: chest drainage, extracorporeal membrane oxygenation, high flow oxygen by nasal cannula, inotropic support, positive pressure ventilation and renal replacement therapy. The diagnosis of multisystem inflammatory syndrome in children (MIS-C) and Kawasaki disease were reported as assigned by the clinical care teams and were considered severe if accompanied by one of the aforementioned diagnoses or interventions.[15]

TABLE 1. Baseline Characteristics of Study Participants Who Presented to Emergency Department for Care Who Were Either Discharged or Hospitalized

	All n = 2520	SARS-CoV-2-Negative n = 2132	SARS-CoV-2 Positive n = 388	*P* Value
Age, years, median (IQR)	2.0 (0.8–5.0)	2.0 (0.9–5.0)	1.0 (0.3–7.8)	0.03
Age, n (%)				<0.001
<1 years	711 (28.2)	553 (25.9)	158 (40.72)	
1 to <5 years	1085 (43.1)	986 (46.2)	99 (25.52)	
5 to <12 years	469 (18.6)	385 (18.1)	84 (21.65)	
≥12 years	255 (10.1)	208 (9.8)	47 (12.11)	
Sex male, n (%)	1375 (54.6)	1157 (54.3)	218 (56.2)	0.49
Country, n (%)				<0.001
Canada	1580 (62.7)	1417 (66.5)	163 (42.01)	
United States	625 (24.8)	455 (21.3)	170 (43.81)	
Australia	190 (7.5)	190 (8.9)	0 (0)	
New Zealand	7 (0.3)	7 (0.3)	0 (0)	
Spain	15 (0.6)	11 (0.5)	4 (1.03)	
Argentina	27 (1.1)	21 (1.0)	6 (1.55)	
Costa Rica	50 (2.0)	28 (1.3)	22 (5.67)	
Singapore	20 (0.8)	3 (0.1)	17 (4.38)	
Paraguay	1 (0)	0 (0)	1 (0.26)	
Italy	5 (0.2)	0 (0)	5 (1.29)	
Days from illness onset to ED visit, n (%)				0.02
≤1 day	718 (28.5)	606 (28.4)	112 (28.866)	
2–3 days	734 (29.1)	601 (28.2)	133 (34.278)	
>3days	1067 (42.4)	924 (43.4)	143 (36.856)	
Variants, n (%)				N/A
Wild-type	242 (62.4)	N/A	242 (62.4)	
Alpha	31 (8.0)	N/A	31 (8.0)	
Delta	26 (6.7)	N/A	26 (6.7)	
Omicron	89 (22.9)	N/A	89 (22.9)	
Having any chronic medical conditions (%)	559 (22.2)	485 (22.8)	74 (19.1)	0.11
Hospitalized at or within 14 days of the index ED visit, n (%)	931 (36.9)	741 (34.8)	190 (49.0)	<0.001

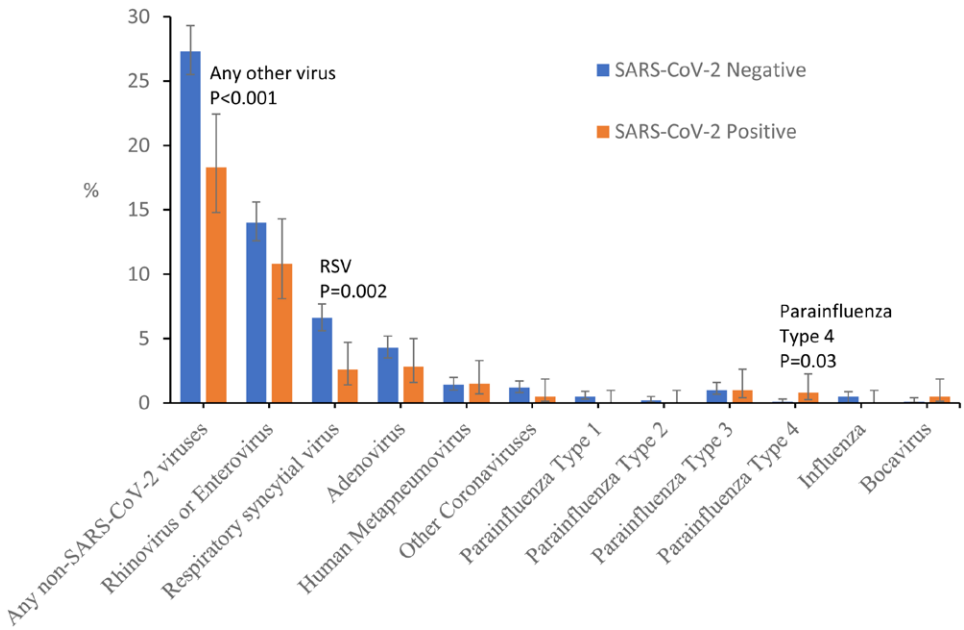

FIGURE 2. Detection rates of non-SARS-CoV-2 respiratory viruses in SARS-CoV-2 positive and negative groups. P values were obtained from χ^2 and Fisher exact test as appropriate for the between SARS-CoV-2-positive and negative groups comparison of the detection rates. The error bars represented 95% CI of the detection rates.

TABLE 2. Unadjusted Analyses for Characteristics Associated with Virus Co-detection Among the 388 SARS-CoV-2-positive Study Participants

	No Additional Virus Detected n = 317	Had Additional Virus(es) Detected n = 71	*P* Value
Age, years, median (IQR)	1.0 (0.3–8.0)	1.0 (0.3–4.0)	0.38
Age, n (%)			0.02
<1 years	131 (41.3)	27 (38.03)	
1 to <5 years	71 (22.4)	28 (39.44)	
5 to <12 years	72 (22.7)	12 (16.90)	
≥12 years	43 (13.6)	4 (5.63)	
Sex male, n (%)	174 (54.9)	44 (62.0)	0.28
Days from illness onset to ED visit, n (%)			0.28
≤1 day	86 (27.1)	26 (36.6)	
2–3 days	111 (35.0)	22 (31.0)	
≥4 days	120 (37.9)	23 (32.4)	
Chronic medical condition, yes (%)	63 (19.9)	11 (15.5)	0.40
Variants			0.03
Wild type	205/317 (64.7)	37/71 (52.1)	
Alpha	23/317 (7.3)	8/71 (11.3)	
Delta	16/317 (5.0)	10/71 (14.1)	
Omicron	73/317 (23.0)	16/71 (22.5)	
SARS-CoV-2 testing date (seasonality)			0.05
2020 March–May	33 (10.41)	2 (2.82)	
2020 June–August	49 (15.46)	6 (8.45)	
2020 September–November	46 (14.51)	12 (16.90)	
2020 December–2021 February	55 (17.35)	10 (14.08)	
2021 March–May	44 (13.88)	14 (19.72)	
2021 June–August	3 (0.946)	1 (1.41)	
2021 September–November	11 (3.47)	7 (9.86)	
2021 December–2022 February	76 (23.974)	19 (26.76)	
Outcomes			
Hospitalization	159 (50.2)	31 (43.7)	0.32
Health care revisit	83 (30.7)	22 (33.8)	0.63
Emergency department revisit	36 (11.4)	10 (14.1)	0.52
Intensive care unit admission	36 (11.4)	7 (9.9)	0.84
Mechanical ventilation	14 (4.4)	3 (4.2)	>0.99
Death	1 (0.3)	0 (0)	>0.99
Severe outcome*			
Any	33 (10.4)	10 (14.1)	0.37
Intensive intervention/treatments	21 (6.6)	3 (4.2)	0.59
Respiratory	12 (3.8)	4 (5.6)	0.51
High flow nasal cannula	12 (3.8)	5 (7.0)	0.21
Kawasaki disease or MIS-C needing intensive intervention or treatments	8 (2.5)	1 (1.4)	>0.99
Cardiac or cardiovascular	5 (1.6)	0 (0)	0.59
Infectious disease	3 (0.9)	1 (1.4)	0.56
Neurologic	3 (0.9)	0 (0)	>0.99

*Composite outcome including the following: cardiac or cardiovascular (cardiac arrest, cardiac ischemia, congestive heart failure, endocarditis, myocarditis, pericarditis and stroke), infectious (disseminated intravascular coagulation, mastoiditis, sepsis with bacteremia, septic shock and shock syndrome), neurologic (encephalitis and meningitis), respiratory (acute respiratory distress syndrome, empyema, necrotizing or cryptogenic organizing pneumonia, pleural effusion or pneumothorax or pneumomediastinum requiring drainage and respiratory failure), any intensive intervention/treatment [chest drainage, extracorporeal membrane oxygenation, inotropic support, positive pressure ventilation (invasive or noninvasive) and renal replacement therapy] or death. One participant in the SARS-CoV-2 positive and 1 in the SARS-CoV-2-negative group had missing data.

of our study (March 2021–Feb 2022), suggesting that co-infection became more common as public health measures were removed.

In a US study that analyzed data collected between March 2020 and February 2022, among 2659 hospitalized SARS-CoV-2 infected children, 21% had a respiratory virus co-detected.[6] That study also found that viral co-infection increased during the delta period. During that same time period, a co-infection rate of just 5.3% was reported among SARS-CoV-2-positive outpatient children in Wisconsin.[8] In a study of children hospitalized in a Turkish hospital between March 2020 and March 2021, only 3.5% of SARS-CoV-2-positive children had an additional respiratory virus detected,[15] while in a primarily outpatient pediatric population (88%) in Switzerland, 11.7% of SARS-CoV-2-positive children had an additional respiratory virus detected.[16] These lower co-infection rates may reflect the impact that early pandemic public health restrictions had on controlling the spread of typical seasonal respiratory viruses.[15,17–19] Our co-detection rate (18%) aligns with Westbrook et al's[1] report of 17% among their subset of children tested in EDs. Interestingly, this rate exceeded that among children tested in other locations, including the operating room, intensive care unit and outpatient and inpatient floors. The varied viral co-infection rates reported likely also reflect the impact of study population, sampling strategy and laboratory testing methodologies employed.

Our study is unique in that it focused on children seeking ED care, among whom 37% were hospitalized. It also was not subject to the limitations of many other studies that were performed retrospectively or are database-derived[1,6]; we conducted interviews with all participants to confirm that they either had symptomatic illness or recent direct SARS-CoV-2 exposure. In addition, most prior studies were conducted in a single healthcare system, geographic jurisdiction or country, while ours included sites in 10 countries. In addition, our evaluation of the likelihood of co-infection was able to integrate clinical variables, such as duration of symptoms, that are unavailable or inaccurately recorded in retrospective and database studies.

TABLE 3. Regression Analysis Evaluating the Adjusted Association Between Viral Detection and Occurrence of Severe Outcomes Among the SARS-CoV-2-positive Children

Model 1			Model 2		
	aOR (95% CI)	*P* Value		aOR (95% CI)	*P* Value
Type of viral detection			Type of viral detection		
Had SARS-CoV-2 and at least one other virus detected	1.74 (0.80–3.79)	0.16	Had SARS-CoV-2 and rhinovirus/enterovirus SARS-CoV-2 detected	3.56 (1.49–8.51)	0.004
Only SARS-CoV-2 detected	reference		Only SARS-CoV-2 detected	reference	
Age, per year older	1.09 (1.03–1.15)	0.002	Age, per year older	1.10 (1.04–1.17)	<0.001
Presence of chronic medical condition			Presence of chronic medical condition		
Yes	2.05 (1.02–4.14)	0.05	Yes	2.15 (1.06–4.36)	0.03
No	Reference		No	Reference	

Models included the 43 study participants who had severe outcomes and 345 study participants who did not have severe outcomes.

In general, respiratory viral co-infections are more common in children, especially those <5 years of age, compared to adults.[2,6] Importantly, many pairings occur at a higher frequency than is expected, suggesting that direct or indirect interactions occur between specific viral pathogens.[2] Similarly, SARS-CoV-2-infected children are more likely to have viral respiratory co-infections than their SARS-CoV-2-infected adult household contacts.[8,16] As seasonal respiratory pathogens reemerged, the association between co-infection and severe outcomes has become increasingly important, particularly among those hospitalized.[6,20] However, the economic and clinical value of multiplex respiratory pathogen panel testing remains a topic of debate, with rapid multiplex pathogen panel testing on ED and inpatient children with respiratory tract infections being inconsistently associated with reductions in antibiotic use and length of stay.[21–23] As measures designed to reduce the spread of SARS-CoV-2 are no longer being implemented, clarifying indications for using multiplex pathogen panels has become increasingly important.

In our study, rhinovirus/enterovirus were the most commonly co-detected respiratory viruses. This is in keeping with previous reports,[6,8,15,16,20] which suggest that rhinovirus/enterovirus circulation did not decrease as much as other respiratory viruses during the early phase of the pandemic and that it surged earlier than other viruses.[18] This is supported by a surveillance study showing that rhinoviruses/enteroviruses were the most frequently detected virus in the prepandemic and pandemic periods, across all pediatric age groups, in both the ED and inpatient settings.[24] Moreover, while detection of rhinovirus/enterovirus was less likely between April 2020 and September 2021, detection of these viruses occurred at similar or higher rates between October 2020 and February 2021 than in the prepandemic period.[24] The frequency of rhinovirus/enterovirus detection may be explained by evidence that children are a major reservoir for rhinovirus infection and are a key driver of transmission to adults.[25] Additionally, it is more transmissible than other viruses (eg, influenza and SARS-CoV-2), even when surgical masks are worn.[26]

Although rhinovirus usually causes mild upper respiratory tract symptoms and can be detected in asymptomatic children,[27] some studies have reported that isolated pediatric rhinovirus/enterovirus infection can be severe,[28] even more severe than RSV and influenza infections.[29,30] Rhinovirus is the second most commonly identified virus in children with bronchiolitis, and it is the virus most commonly co-detected alongside RSV.[31] Our finding that the detection of rhinovirus/enterovirus in children with SARS-CoV-2 was associated with an increased odds of severe outcomes supports the similar finding reported by Agathis et al.[6,20] These findings highlight the importance of ongoing monitoring of viral infections and co-infection in children to inform strategies to prevent and mitigate the impact on children and the health care system. Moreover, enhanced respiratory virus surveillance in children, particularly those who are severely unwell, is crucial. Our findings should inform infection control practices in healthcare settings, by highlighting the importance of stringent isolation protocols to prevent the spread of respiratory viruses and the development of nosocomial co-infections. Finally, public health strategies should prioritize vaccination efforts for preventable respiratory infections (eg, influenza, SARS-CoV-2 and RSV) in children.

Currently, we have a limited understanding of the mechanism by which SARS-CoV-2 and concomitant viral co-infections lead to more severe outcomes. An in vitro study suggested the delta variant upregulated α-2-3-linked sialic acid, while influenza upregulated angiotensin-converting enzyme 2 and transmembrane serine protease 2.[32] In the same model, SARS-CoV-2 and influenza co-infection caused greater hyperactivation of proinflammatory and immune-related signaling pathways and cellular damage compared to single virus infection. In addition, preinfection with influenza strongly enhances the infectivity of SARS-CoV-2 by boosting viral entry into cells and co-infection leads to elevated SARS-CoV-2 viral loads and more severe lung damage in infected mice.[33]

Limitations

Our study has several limitations that should be considered when interpreting the findings. First and most importantly, as has been reported by others,[6,34] testing for viral co-infection and the multiplex panels employed were based on physician decision-making and institutional practice and policies. This introduced selection bias as hospitalized children and those with more severe illnesses were more likely to undergo expanded multiplex testing. Overrepresentation of more severe cases with a higher likelihood of detecting viruses such as rhinovirus/enterovirus, which may be less prevalent in less severely ill children, may have led to an overestimate of the association between viral co-infection and severe outcomes. Additionally, at institutions where multiplex assays are limited to SARS-CoV-2, influenza and RSV, other viruses would not have been detected, potentially leading to different clinical conclusions. We unfortunately do not have documentation of which participants were tested for which viruses.

Although our analysis was restricted to children who had additional viral testing performed, residual biases may remain. As has been reported by others,[6] we do not know the timing of the infections and cannot attribute, with certainty, the viruses identified to the clinical outcomes observed. Due to the low detection rate of viruses other than rhinovirus/enterovirus, we were underpowered to study the impact that co-infection of these viruses had on patient outcomes. In addition, as nucleic acid amplification tests can detect SARS-CoV-2 RNA weeks to months after initial infection,[35] in

some participants, a positive SARS-CoV-2 test may represent shedding from recent infection and may not be etiologic of presenting symptoms. As such, when viruses are co-detected, we cannot state with certainty that they are both causal of the symptoms or clinical outcomes. Finally, as SARS-CoV-2 variants evolve over time, the findings may not be generalizable to emerging variants. Thus, while our findings contribute to understanding the clinical impact of viral co-infections in SARS-CoV-2-infected children, they should be interpreted with caution.

CONCLUSIONS

We found that although the detection of additional respiratory viruses in SARS-CoV-2-infected children was not uncommon, those with rhinovirus/enterovirus co-infections were more likely to experience severe outcomes. Thus, viral co-infection should be considered in children with SARS-CoV-2 infection who present with severe illness requiring hospitalization, with rhinovirus/enterovirus being the most commonly co-detected respiratory viruses. As the co-infection of SARS-CoV-2 and other respiratory viruses has increased, it remains important to monitor the clinical impact of co-infections to inform clinical decision-making and public health measures.

T.F. reported receiving grants from the National Institute of Allergy and Infectious Diseases and the National Heart, Lung, and Blood Institute, and serving as a speaker for Diasorin, all outside the submitted work. L.A. reported receiving grants from Pfizer outside the submitted work. R.M. reported receiving personal fees from Merck as a consultant, grants from Pfizer, grants from Astellas and reported being a board member and consultant for Affinivax Scientific Founder outside the submitted work. C.R.M. reported royalties from intellectual property from UCSF Benioff Children's Hospital Oakland; grants from NIH/NHLBI, grants from FDA, and grants from NIH/NCCIM outside the submitted work; in addition, C.R.M. had a patent for therapeutics related to Arginine bioavailability in COVID-19 and MIS-C pending. S.M.B. reported receiving Primary Children's Hospital Fee per enrolled study participant from the University of Utah during the conduct of the study. N.K. reported receiving grants from the National Institute of Child Health and Human Development, the Health Resources and Services Administration, the Centers for Disease Control and Prevention, and the Patient-Centered Outcomes Research Institute, all outside the submitted work. Other authors have no conflicts of interest to disclose.

ACKNOWLEDGMENTS

We acknowledge the many research professionals across the globe working in PERC and PERN emergency departments who enrolled study participants and managed research processes. We are especially grateful to all the study participants and families who participated in the PERC and PERN-COVID-19 studies. We acknowledge our collaborators including Dr. Terry Klassen (University of Manitoba), Dr. Santiago Mintegi and Dr. Iker Gangoiti (University of the Basque Country), UPV/EHU. Bilbao, Basque Country, Spain for their contributions to this study.

Pediatric Emergency Research Network (PERN)-COVID-19 Study Team: Kelly R. Bergmann, Jasmine R. Nebhrajani, Meredith L. Borland, Fahd A. Ahmad, Stuart R. Dalziel, Richard Malley, Usha R. Avva, Kristen A. Breslin, Nipam P. Shah, Adriana Yock-Corrales, Sarah M Becker, Maren M. Lunoe, Claudia R. Morris, Alexander J. Rogers, Pradip P. Chaudhari, Shu-Ling Chong, Usha Sethuraman, Pedro B. Rino, Kerry Caperell, Michael A. Gardiner, Mark I. Neuman, Nidhya Navanandan, Laura Palumbo, Maria Y. Kwok, Carmen Campos, Laura F. Sartori, Viviana Pavlicich, Andrea K. Morrison, Michelle Eckerle, Muhammad Waseem, Carlos Miguel Angelats Romero, Marina I. Salvadori, Lilliam Ambroggio, and Daniel C. Payne.

Pediatric Emergency Research Canada (PERC)-COVID-19 Study Team: Kelly Kim, Kathleen Winston, Darcy Beer, Jocelyn Gravel, April J. Kam, Robert Porter, Amy C. Plint, Ahmed Mater, Vikram J. Sabhaney, Maala Bhatt, Roger Zemek, Brett Burstein, Yaron Finkelstein, Bruce Wright, Jonathan C. Cherry, Anne Moffatt, Simon Berthelot, Gabrielle Freire, and Naveen Poonai.

REFERENCES

1. Westbrook A, Wang T, Bhakta K, et al. Respiratory coinfections in children with SARS-CoV-2. *Pediatr Infect Dis J*. 2023;42:774–780.
2. Mandelia Y, Procop GW, Richter SS, et al. Dynamics and predisposition of respiratory viral co-infections in children and adults. *Clin Microbiol Infect*. 2021;27:631.e1–631.e6.
3. Asner SA, Science ME, Tran D, et al. Clinical disease severity of respiratory viral co-infection versus single viral infection: a systematic review and meta-analysis. *PLoS One*. 2014;9:e99392.
4. Li Y, Pillai P, Miyake F, et al. The role of viral co-infections in the severity of acute respiratory infections among children infected with respiratory syncytial virus (RSV): a systematic review and meta-analysis. *J Glob Health*. 2020;10:010426.
5. Guerrier G, Goyet S, Chheng ET, et al. Acute viral lower respiratory tract infections in Cambodian children: clinical and epidemiologic characteristics. *Pediatr Infect Dis J*. 2013;32:e8–13.
6. Agathis NT, Patel K, Milucky J, et al; CDC COVID-NET Surveillance Team. Codetections of other respiratory viruses among children hospitalized with COVID-19. *Pediatrics*. 2023;151:e2022059037.
7. Kim D, Quinn J, Pinsky B, et al. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *JAMA*. 2020;323:2085–2086.
8. Temte JL, Barlow S, Temte E, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) codetection with influenza A and other respiratory viruses among school-aged children and their household members-12 March 2020 to 22 February 2022, Dane County, Wisconsin. *Clin Infect Dis*. 2022;75:S205–S215.
9. Klassen TP, Acworth J, Bialy L, et al; PERN. Pediatric emergency research networks: a global initiative in pediatric emergency medicine. *Pediatr Emerg Care*. 2010;26:541–543.
10. Bialy L, Plint A, Zemek R, et al; Pediatric Emergency Research Canada (PERC). Pediatric emergency research Canada: origins and evolution. *Pediatr Emerg Care*. 2018;34:138–144.
11. Funk AL, Florin TA, Dalziel SR, et al. Prospective cohort study of children with suspected SARS-CoV-2 infection presenting to paediatric emergency departments: a Paediatric Emergency Research Networks (PERN) Study Protocol. *BMJ Open*. 2021;11:e042121.
12. Funk AL, Florin TA, Kuppermann N, et al; Pediatric Emergency Research Network-COVID-19 Study Team. Outcomes of SARS-CoV-2-positive youths tested in emergency departments: the global PERN-COVID-19 study. *JAMA Netw Open*. 2022;5:e2142322.
13. Monegro AF, Muppidi V, Regunath H. Hospital-Acquired Infections. [Updated 2023 Feb 12]. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Available at: https://www.ncbi.nlm.nih.gov/books/NBK441857/.
14. Sumner MW, Xie J, Zemek R, et al; Pediatric Emergency Research Canada (PERC) COVID Study Group. Comparison of symptoms associated with SARS-CoV-2 variants among children in Canada. *JAMA Netw Open*. 2023;6:e232328.
15. Kıymet E, Böncüoğlu E, Şahinkaya S, et al. Distribution of spreading viruses during COVID-19 pandemic: effect of mitigation strategies. *Am J Infect Control*. 2021;49:1142–1145.
16. Pigny F, Wagner N, Rohr M, et al; Geneva Pediatric COVID Group. Viral co-infections among SARS-CoV-2-infected children and infected adult household contacts. *Eur J Pediatr*. 2021;180:1991–1995.
17. Olsen SJ, Winn AK, Budd AP, et al. Changes in influenza and other respiratory virus activity during the COVID-19 pandemic-United States, 2020-2021. *Am J Transplant*. 2021;21:3481–3486.
18. Rodgers L, Sheppard M, Smith A, et al. Changes in seasonal respiratory illnesses in the United States during the coronavirus disease 2019 (COVID-19) pandemic. *Clin Infect Dis*. 2021;73:S110–S117.

19. Yeoh DK, Foley DA, Minney-Smith CA, et al. Impact of coronavirus disease 2019 public health measures on detections of influenza and respiratory syncytial virus in children during the 2020 Australian Winter. *Clin Infect Dis*. 2021;72:2199–2202.
20. Dikranian L, Barry S, Ata A, et al; From the Society of Critical Care Medicine Discovery Viral Infection and Respiratory Illness Universal Study: COVID-19 Registry Investigator Group. SARS-CoV-2 with concurrent respiratory viral infection as a risk factor for a higher level of care in hospitalized pediatric patients. *Pediatr Emerg Care*. 2022;38:472–476.
21. May L, Tatro G, Poltavskiy E, et al. Rapid multiplex testing for upper respiratory pathogens in the emergency department: a randomized controlled trial. *Open Forum Infect Dis*. 2019;6:ofz481.
22. Kitano T, Nishikawa H, Suzuki R, et al. The impact analysis of a multiplex PCR respiratory panel for hospitalized pediatric respiratory infections in Japan. *J Infect Chemother*. 2020;26:82–85.
23. Mattila S, Paalanne N, Honkila M, et al. Effect of point-of-care testing for respiratory pathogens on antibiotic use in children: a randomized clinical trial. *JAMA Netw Open*. 2022;5:e2216162.
24. Rankin DA, Spieker AJ, Perez A, et al; NVSN Network Investigators. Circulation of rhinoviruses and/or enteroviruses in pediatric patients with acute respiratory illness before and during the COVID-19 pandemic in the US. *JAMA Netw Open*. 2023;6:e2254909.
25. Poole S, Brendish NJ, Tanner AR, et al. Physical distancing in schools for SARS-CoV-2 and the resurgence of rhinovirus. *Lancet Respir Med*. 2020;8:e92–e93.
26. Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med*. 2020;26:676–680.
27. Kieninger E, Fuchs O, Latzin P, et al. Rhinovirus infections in infancy and early childhood. *Eur Respir J*. 2013;41:443–452.
28. Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. 2019;394:757–779.
29. Asner SA, Petrich A, Hamid JS, et al. Clinical severity of rhinovirus/enterovirus compared to other respiratory viruses in children. *Influenza Other Respir Viruses*. 2014;8:436–442.
30. Miller EK, Lu X, Erdman DD, et al; New Vaccine Surveillance Network. Rhinovirus-associated hospitalizations in young children. *J Infect Dis*. 2007;195:773–781.
31. Kenmoe S, Kengne-Nde C, Ebogo-Belobo JT, et al. Systematic review and meta-analysis of the prevalence of common respiratory viruses in children < 2 years with bronchiolitis in the pre-COVID-19 pandemic era. *PLoS One*. 2020;15:e0242302.
32. Kim MJ, Kim S, Kim H, et al. Reciprocal enhancement of SARS-CoV-2 and influenza virus replication in human pluripotent stem cell-derived lung organoids(1). *Emerg Microbes Infect*. 2023;12:2211685.
33. Bai L, Zhao Y, Dong J, et al. Coinfection with influenza A virus enhances SARS-CoV-2 infectivity. *Cell Res*. 2021;31:395–403.
34. Swets MC, Russell CD, Harrison EM, et al; ISARIC4C Investigators. SARS-CoV-2 co-infection with influenza viruses, respiratory syncytial virus, or adenoviruses. *Lancet (London, England)*. 2022;399:1463–1464.
35. Wajnberg A, Mansour M, Leven E, et al. Humoral response and PCR positivity in patients with COVID-19 in the New York City region, USA: an observational study. *Lancet Microbe*. 2020;1:e283–e289.