

1 Virome of New York Presbyterian Hospital 2 pediatric emergency

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6 Abstract

7 Background

8 Viral infection of the respiratory tract is one of the major causes of hospital visits for young
9 children. In this study, we report the occurrence and co-occurrence of different virus types and
10 subtypes among the patients arriving at the pediatric emergency room of New York Presbyterian
11 Hospital, a major urban hospital.

12 Methods

13 We collected nasal swabs from the patients and their accompanying persons. We also recorded the levels
14 (None, Mild, High, and Severe) of their symptoms (Fever, Chill, Muscle Pain, Watery Eye, Runny Nose,
15 Sneezing, Sore throat and Cough). The collected swabs were tested for the presence of common viruses
16 infecting the respiratory tract.

17 Results

18 Human Rhinovirus was the most common virus among the patients, followed Influenza and Respiratory
19 Syncytial Virus. Human Rhinovirus was most common in summer, autumn and spring. In contrast,
20 influenza was more common in winter. Further, Influenza A virus was more likely to co-occur with
21 Corona Virus 229E. In comparison, Influenza was less likely to co-occur with Human Rhinovirus.
22 Moreover, Influenza, Parainfluenza and Corona virus were associated with more severe symptoms, while

1 Human Rhinovirus was associated with less severe symptoms. In addition, we observed that Influenza
2 and Respiratory Syncytial Virus were more likely to infect a patient when these viruses also infected the
3 accompanying person. We also found that it was difficult to distinguish among viruses based on the
4 symptoms. The inability to distinguish among different virus types and subtypes is explained by the fact
5 that multiple viruses lead to similar symptoms.

6 Conclusions

7 The findings of this study provide a better understanding of respiratory viral infections in small children
8 presenting at a pediatric emergency room in New York.

9

10 Key Words

11 Respiratory Tract, Viral Infection, Coinfection, Cross Infection, Conditional Infection, Symptoms,
12 Seasonality, Transmission in Family, Respiratory Emergency

13

14 Background

15 Respiratory Tract Infections (RTIs) are among the most common illnesses irrespective of age [1], and a
16 major cause of child mortality [2, 3]. Viral RTI spread more readily among small children, whose lifetime
17 exposure to these pathogens is more limited and whose immune systems may be less developed. Previous
18 large-scale studies in both children and adults have demonstrated differences among viral RTI agents in
19 terms of infection rates, seasonality and severity of symptoms [4-6]. Additionally, the prevalence of co-
20 infection by multiple viruses has also been reported in certain settings [7]. However, the incidence
21 correlation of viral infections as well as the asymptomatic infection rates in family members of infected
22 children remains poorly understood.

1 In this study, nasal swabs and symptomology were collected from patients arriving at the pediatric
2 emergency room (PedER) of New York Presbyterian Hospital (NYPH). Swabs were assayed for the
3 presence of different viral types and subtypes. Here we report the number of patients infected by each
4 virus, the interdependence of viral infections expressed as conditional infection rates i.e. whether
5 infection by a certain virus makes a patient more likely to be infected by another virus, shared
6 symptomatic and asymptomatic infection rates among accompanying family members, and the
7 differences in symptoms among different viruses.

8

9 Methods

10 In this cross-sectional study, participants were enrolled between 1 Aug 2016 and 4 Aug 2017 at the
11 pediatric emergency department of NYPH, a single, urban pediatric emergency department with an
12 annual volume of approximately 50,000 patients. Children below the age of 18 presenting with respiratory
13 complaints (i.e. acute illness, asthma) and their accompanying adult (e.g. parents or other relatives) were
14 provided a detailed study description and if interested, provided consent to participate. The consenting
15 adult filled out a questionnaire for themselves and the child, and two nasopharyngeal samples were
16 collected from each participant (accompanying adult and the child with complaints).

17 Questionnaire

18 The questionnaire included information on ethnicity, general health (i.e. if they often feel sick), daily
19 habits, travel history in the previous month and household structure. Nine respiratory illness-related
20 symptoms (fever, chills, muscle pain, watery eyes, runny nose, sneezing, sore throat, cough, chest pain
21 during the past 48 hours) were also recorded on a Likert scale (0=none, 1=mild, 2=moderate, 3=severe).

1 Specimen collection and analysis

2 From each participant two nasopharyngeal samples were collected using minitip flocked swabs. Both the
3 samples were stored in a single tube in 2ml DNA/RNA Shield (Zymo Research, Irvine, CA) at 4-25°C for
4 up to 30 days. The samples were then split into two aliquots, and stored at -80°C. Total nucleic acid
5 extraction was completed using the easyMAG NucliSENS (Biomerieux, Durham, NC). The eSensor XT-
6 8 Respiratory Viral Panel (RVP; GenMark Diagnostics, Carlsbad, CA), a multiplex PCR assay, was used
7 for virus detection according to the manufacturer's instructions. The RVP separately detects influenza
8 (Inf) A (any subtype: A/H1N1, A/H3N2, A/H1N1pdm2009), influenza B, Respiratory Syncytial Virus
9 (RSV) A and B, parainfluenza (PIV) 1, 2, 3, and 4, human metapneumovirus (hMPV), human rhinovirus
10 (HRV), adenovirus B/E and C, and coronavirus (CV) 229E, NL63, OC43, and HKU1. The eSensor
11 system measures electrical current density in nA/mm². Samples that produced current density ≥ 2 nA/mm²
12 for a particular virus were considered positive except for CV OC43. According to protocol of GenMark,
13 the threshold for considering a sample positive for CV OC43 was 25 nA/mm².

14 We used numpy and scipy statistics packages to calculate mean symptom scores, the coinfection rates (i.e.
15 fraction of patients infected simultaneously by two viruses), conditional infection rates and statistical
16 significances from the data. The mean symptom scores were calculated by taking the arithmetic mean of
17 the symptom scores, and coinfection rates were calculated by dividing the number of coinfections by the
18 total number of patients. The conditional infection rate of any virus A given infection by virus B was
19 calculated by dividing the number of A and B coinfections by the number of infection by virus B, and
20 finally we used Fisher's exact test with Bonferroni correction to estimate the statistical significance of the
21 conditional infection rates.

1 Results

2 Patients characteristics

3 340 patients (i.e. children below age 18 years presenting at the PedER) between the ages of 2 months and
4 18 years (mean ~6 years) were enrolled between 1 Aug 2016 and 4 Aug 2017. The number of adults
5 accompanying the patients was 324. The majority (62%) of patients were <5 years old. There were
6 included 174 male, 165 female and 1 transgender patient. Number of patients varied per season: summer,
7 67 patients; autumn, 91 patients; winter, 89 patients; and spring, 92 patients. The number of patients
8 shedding each virus is depicted in Table 1. HRV was the most common virus followed by influenza (all
9 types) and respiratory RSV B.

10

Virus	Total	Summer	Autumn	Winter	Spring
Inf	47	0	4	29	14
Inf A	29	0	1	26	2
Inf B	18	0	3	3	12
RSV A	17	0	4	13	0
RSV B	18	0	6	10	1
PIV 1	12	7	0	4	1
PIV 2	10	1	7	2	0
PIV 3	17	6	2	0	9
PIV 4	3	3	0	0	0
hMPV	15	2	4	6	3
HRV	84	12	37	7	27
Adeno B	5	0	3	1	1
Adeno C	15	2	7	3	3

CV 229E	10	0	1	7	2
CV NL63	1	0	0	0	1
CV HKU1	2	0	0	0	2
CV OC43	5	0	0	2	3

1 Table 1: Number of patients shedding different virus.

2 Well-known seasonal patterns were observed. For example, RSV A, RSV B and Inf A were most
 3 common in winter, whereas influenza B and PIV 3 were most common in spring. HRV occurred all year,
 4 but was more common in autumn and spring (Table 1**Error! Reference source not found.**). Note that
 5 due to the ambiguity in determining whether a person shedding a virus is infected or not, in this study we
 6 use the terms ‘shedding a virus’ and ‘infected by a virus’ interchangeably.

7 Infection rates are dependent on viral interactions

8 The infection rates of different viruses were correlated with infection by other viruses (Table 2). For
 9 instance, 8% of all patients were infected by Inf A**Error! Reference source not found.**; however, 50 %
 10 of the patients infected by CV 229E were also infected by Inf A (i.e. the conditional infection rate of Inf
 11 A given CV 229E was significantly different from random chance, $P < 0.001$ Bonferroni corrected Fisher’s
 12 exact test). The most striking feature of the conditional infection rates was the almost complete exclusion
 13 between Inf and HRV. HRV (24%) and Inf A (8%) were both very common infections among the
 14 subjects. Thus, one would expect the number of patients coinfecting by both HRV and Inf A to be
 15 approximately 2% of the patients; however, the observed coinfection rate was close to zero ($P < 0.01$,
 16 Bonferroni corrected Fisher’s exact test).

	Inf	InfA	InfB	RSV A	RSV B	PIV 3	hMPV	HRV	Adeno C
Count	47	29	18	17	18	17	15	84	15

Infection rate	0.14	0.08	0.05	0.05	0.05	0.05	0.04	0.24	0.04
Inf		0.62	0.38	0.00	0.02	0.00	0.00	0.02	0.04
Inf A			0.00	0.00	0.03	0.00	0.00	0.00	0.03
Inf B		0.00		0.00	0.00	0.00	0.00	0.06	0.06
RSV A	0.00	0.00	0.00		0.00	0.06	0.00	0.06	0.06
RSV B	0.06	0.06	0.00	0.00		0.00	0.00	0.00	0.06
PIV 1	0.08	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PIV 2	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.50	0.00
PIV 3	0.00	0.00	0.00	0.06	0.00		0.06	0.18	0.06
PIV 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00
hMPV	0.00	0.00	0.00	0.00	0.00	0.07		0.07	0.00
HRV	0.01	0.00	0.01	0.01	0.00	0.04	0.01		0.05
Adeno B	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.20	0.40
Adeno C	0.13	0.07	0.07	0.07	0.07	0.07	0.00	0.27	
CV 229E	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.10	0.00
CV NL63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CV HKU1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50

1 Table 2: Conditional infection rates: The count row indicates the number of observed cases for each virus in the columns. The
2 numbers in the infection rates row are calculated by dividing the count by the number of patients. The rows with virus names in
3 the leftmost column indicate the conditional infection rates of the virus in the column given infection by the virus in the row.
4 Only viruses presenting at least 15 counts are shown in this table. Rates significantly different from expected are shown in bold.

5 Infection in the Family

6 Having an infected family member increased the chance of infection by the same virus up to ten times
7 (Fig. 1). Compared to the infection rates among all patients (i.e. fraction of all patients shedding a virus),

1 the infection rates among patients with infected family member(s) were significantly different ($P < 0.001$)
 2 for Inf A, Inf B, RSV A, and RSV B as tested by Bonferroni corrected Fisher's exact test.
 3
 4 As shown in table 3, some of the infected family members were asymptomatic. The rate of asymptomatic
 5 infection was highest for HRV followed by RSV and Inf.

	Definition 1		Definition 2		Definition 3		Definition 4	
	Count	Rate	Count	Rate	Count	Rate	Count	Rate
Inf	12	0.037037	14	0.04321	14	0.04321	7	0.021605
InfA	5	0.015432	7	0.021605	7	0.021605	2	0.006173
InfB	7	0.021605	7	0.021605	7	0.021605	5	0.015432
RSV A	7	0.021605	11	0.033951	12	0.037037	6	0.018519
RSV B	10	0.030864	10	0.030864	10	0.030864	10	0.030864
PIV 1	4	0.012346	4	0.012346	4	0.012346	4	0.012346
PIV 2	3	0.009259	3	0.009259	3	0.009259	3	0.009259
PIV 3	5	0.015432	5	0.015432	5	0.015432	3	0.009259
PIV 4	1	0.003086	1	0.003086	1	0.003086	1	0.003086
hMPV	2	0.006173	2	0.006173	2	0.006173	2	0.006173
HRV	31	0.095679	33	0.101852	34	0.104938	30	0.092593
Adeno B	2	0.006173	2	0.006173	2	0.006173	1	0.003086
Adeno C	3	0.009259	3	0.009259	4	0.012346	2	0.006173
CV 229E	1	0.003086	4	0.012346	4	0.012346	1	0.003086

6
 7 Table. 3: Asymptomatic infection rates of different viruses among the companions of the patients. The asymptomatic infection
 8 rate is calculated by dividing the number of asymptomatic infections by the number of companions. An asymptomatic infection is
 9 defined in 4 different ways. Definition 1: total symptoms score less than 5, definition 2: total symptoms score less than 10,
 10 definition 3: total symptoms score less than 15, and definition 4: none of the 9 recorded symptoms is severe (i.e. score 3).

1 Knowledge of seasonal viruses improves symptomatic diagnosis

2 The symptoms experienced varied by virus and season. As shown in Fig. 2, influenza (both A and B)
3 caused severe fever in autumn, but more moderate fever in winter and spring. In contrast, PIV 2 caused
4 severe fever in summer but mild fever during winter and spring. HRV was associated with rather mild
5 fever in all seasons. RSV A and RSV B produced more severe fever during autumn but milder fever
6 during winter.

7 Diagnosing the causative agent of RTI from symptoms is challenging due to the similarity of the
8 symptoms exhibited by different respiratory viruses. However, despite the similarity of symptoms,
9 knowledge of background infection rates and severity of specific symptoms was useful for diagnosing
10 viral infection type. As an example, we compared the likelihood of infection by Influenza (both A and B)
11 and HRV. Among patients year-round, the infection rate of Inf was 14% and that of HRV was 24% , but
12 among patients exhibiting moderate to severe muscle pain Inf was more likely (29%) than HRV (21%).
13 In contrast, patients with moderate to severe chest pain had a 33% chance of HRV infection compared to
14 an 8% chance of Inf. However, using models to predict of infection based on the symptoms taking into
15 account overall infection rates for the past 4 weeks were ineffective in distinguishing different viruses.
16 This indicated that accurate diagnosis of a specific respiratory infectious agent by symptoms is limited.

17 Discussion

18 Our findings in this study of patients at the NYPH PedER are consistent with previous reports of seasonal
19 viral infections and varying degrees of severity of symptoms based on the virus [4-6, 8]. Moreover, this
20 study also provides data on the likelihood of infection by a given virus when patients exhibit different
21 symptoms. Moreover, the most frequently reported viruses (HRV, Inf, RSV, ADV and PIV) from this
22 study matches with those from Wang et al. [6]; however, the most prevalent virus in this study was HRV
23 rather than RSV as reported by Wang et al. [6].

24

1 In this study, we found that influenza, parainfluenza and corona virus were associated with more severe
2 symptoms. Among the symptoms fever, runny nose, cough and sneeze were frequent while chest pain,
3 muscle pain and watery eye were less common. However, symptom severity varied seasonally for a given
4 virus. Seasonality of different viruses among the subjects of this study followed the well-known seasonal
5 patterns. For instance, in the overall yearly data, HRV was most common followed by Inf A and RSV.
6 However, these viruses had very different seasonal prevalence. HRV was most common in summer,
7 autumn and spring with influenza being most common in winter.

8
9 Our finding suggested that Inf B, RSV A, PIV 1, hMPV, and Adeno B were more likely to infect
10 children when the accompanying family members were also infected. Additionally, more than 10% of the
11 accompanying family members were infected by respiratory viruses despite showing no symptoms.

12
13 Examination of conditional infection rates indicates viral interaction. In particular influenza infection was
14 more common among patients with CV 229E and appeared to be inhibited among patients with HRV.
15 The exact reason behind this is not clear. The present study is limited by the number of patients. Further
16 study of such viral facilitation and inhibition with larger sample sizes might clarify this interesting
17 phenomenon.

18

19 Conclusions

20 This study provides insights into occurrence, co-occurrence and asymptomatic infection rates of
21 respiratory viruses in New York. The results indicated that HRV, RSV and Inf were the most common
22 viruses in pediatric emergency setting. Despite both being common viruses, HRV and Inf coinfection was
23 rare. Moreover, Inf B, RSV A, PIV 1, hMPV, and Adeno B were more likely to infect children when the
24 accompanying family members were also infected. Additionally, around 10% of the accompanying adults
25 were asymptotically infected by respiratory viruses. These findings would facilitate future treatment
26 and control of respiratory viral infections.

1 List of Abbreviation

2 CV Corona Virus

3 hMPV Human Metapneumovirus

4 HRV Human Rhinovirus

5 Inf Influenza

6 Inf A Influenza A

7 Inf B Influenza B

8 IRB Institutional Review Board

9 NYPH New York Presbyterian Hospital

10 PedER Pediatric Emergency Room

11 PIV Parainfluenza Virus

12 RSV Respiratory Syncytial Virus

13 RTI Respiratory Tract Infections

14 RVP Respiratory Viral Panel

15 Declarations

16 Ethics approval and consent to participate

17 The study was approved by the institutional review board (IRB) of Columbia University. The IRB
18 approval number is AAAQ4358.

19 Consent for publication

20 Not Applicable.

1 Availability of data and material

2 The datasets generated and/or analyzed during the current study are not publicly available due to patient
3 privacy but are available from the corresponding author on reasonable request.

4 Competing interests

5 The authors declare that they have no competing interests.

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10 government.

11 Authors' contributions

12 JLS, GF and PD designed the study. JLS, HM, SI and PD oversaw collection of samples and
13 questionnaires. DC, SI, BL, CL, NM, AS and ET analyzed the samples. MU and IF analyzed data. MU,
14 JLS, PD, IF, MG, RB, HM and DC wrote the paper.

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20 **epidemic situations in Shanghai.** *Journal of Clinical Virology* 2010, **49**:211-218.

21 Figure Legends

22

23 Fig 1. Likelihood a patient is infected by the same virus as an accompanying family member

24 (orange) versus the overall pool of patients (blue).

1 Fig. 2: Average fever score associated with viral infections during different seasons as
2 measured according to the self-reported scale varying from 0 to 3.

3

4



