Original Article



Air dispersal of respiratory viruses other than severe acute respiratory coronavirus virus 2 (SARS-CoV-2) and the implication on hospital infection control

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Abstract

Background: Air dispersal of respiratory viruses other than SARS-CoV-2 has not been systematically reported. The incidence and factors associated with air dispersal of respiratory viruses are largely unknown.

Methods: We performed air sampling by collecting 72,000 L of air over 6 hours for pediatric and adolescent patients infected with parainfluenza virus 3 (PIF3), respiratory syncytial virus (RSV), rhinovirus, and adenovirus. The patients were singly or 2-patient cohort isolated in airborne infection isolation rooms (AIIRs) from December 3, 2021, to January 26, 2022. The viral load in nasopharyngeal aspirates (NPA) and air samples were measured. Factors associated with air dispersal were investigated and analyzed.

Results: Of 20 singly isolated patients with median age of 30 months (range, 3 months–15 years), 7 (35%) had air dispersal of the viruses compatible with their NPA results. These included 4 (40%) of 10 PIF3-infected patients, 2 (66%) of 3 RSV-infected patients, and 1 (50%) of 2 adenovirus-infected patients. The mean viral load in their room air sample was 1.58×10^3 copies/mL. Compared with 13 patients (65%) without air dispersal, these 7 patients had a significantly higher mean viral load in their NPA specimens (6.15×10⁷ copies/mL vs 1.61×10^5 copies/mL; *P* < .001). Another 14 patients were placed in cohorts as 7 pairs infected with the same virus (PIF3, 2 pairs; RSV, 3 pairs; rhinovirus, 1 pair; and adenovirus, 1 pair) in double-bed AIIRs, all of which had air dispersal. The mean room air viral load in 2-patient cohorts was significantly higher than in rooms of singly isolated patients (1.02×10^4 copies/mL vs 1.58×10^3 copies/mL; *P* = .020).

Conclusion: Air dispersal of common respiratory viruses may have infection prevention and public health implications.

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The transmission of respiratory viruses by droplet or contact routes in healthcare settings is a principal dogma of infection prevention. The transmission-based precautions as illustrated in the recommendations for isolation precautions in hospitals by the Centers for Disease Control and Prevention (CDC) were based on this dogma since 1996.^{1,2} However, transmission of respiratory viruses by airborne route has been implicated in community settings over the past decades, including transmission of influenza

A in a commercial airliner³ or within household,⁴ and transmission of rhinovirus among game card players.⁵ Spread of respiratory syncytial virus (RSV) by aerosol was also suggested in the healthcare setting.⁶ During the outbreak of severe acute respiratory syndrome (SARS) in 2003 by SARS coronavirus 1 (SARS-CoV-1), airborne transmission of SARS-CoV-1 was observed in both community and healthcare settings.^{7,8} With the emergence of coronavirus disease 2019 (COVID-19) due to SARS coronavirus 2 (SARS-CoV-2), airborne transmission has been increasingly reported in the healthcare and community settings.⁹⁻¹⁴

Since we have been performing air sampling to detect of SARS-CoV-2 RNA in the airborne infection isolation room (AIIR) of hospitals and community treatment facilities during the COVID-19 pandemic,^{15–18} we would like to know whether air dispersal also occurs in patients infected with common respiratory viruses other than SARS-CoV-2. Here, we performed room air

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Fig. 1. The floor plan of a pediatric ward of Queen Mary Hospital. Note. The pediatric ward contains 28 beds in 6 double-bed airborne infection isolation rooms (AIIRs) (bed numbers 1, 1A, 2, 2A, 3, 3A, 4, 4A, 5, 5A, 6, and 6A), 1 single-bed room (bed number 7), and three 5-bed cubicles (bed numbers 8–22) without pressure difference between the cubicles and the common area. The air sampler is denoted as a red rectangle placed at the corner of the AIIRs at a distance >2 m from the patient's head.

sampling of pediatric and adolescent patients with laboratory-confirmed respiratory viral infection. These findings may have implications in infection prevention and public health measures.

Methods

Setting

This study was conducted in a pediatric ward of Queen Mary Hospital, a 1,700-bed, university-affiliated, teaching hospital in Hong Kong. The pediatric ward contains 28 beds arranged as 6 double-bed AIIRs (room 1–6), 1 single-bed (room 7), and three 5-bed cubicles (room 8–10) without pressure difference between the cubicles and the common area (Fig. 1). The air changes per hour in the AIIRs and the cubicles are 12 and 6, respectively. The temperature and humidity of the AIIRs are set at 22°C and 65%, respectively. The AIIR is prioritized to care for patients aged \leq 17 years and infected with pathogens of airborne transmission. Other patients who are aged \leq 17 years and with fever and respiratory symptoms will also be admitted through the emergency department to this pediatric ward. This study was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Hospital Cluster.

Microbiological diagnosis of patients with respiratory symptoms

Upon admission, nasopharyngeal aspirates (NPA) were collected for rapid molecular diagnostic test. The NPA in viral transport medium (VTM) were simultaneously tested for 23 pathogens using the BIOFIRE FILMARRAY Respiratory 2.1 plus Panel (bioMérieux, Marcy l'Étoile, France).¹⁹

Collection of air sample from patients with respiratory viral infection

Patients infected by a single virus detected by BIOFIRE FILMARRAY Respiratory 2.1 plus Panel were eligible for this study. Patients with newly diagnosed respiratory viral infection and singly isolated in AIIRs were selected. If >1 eligible patient was available on the day of air sample collection, only 1 patient was chosen at random. In addition, air sampling in double-bed AIIRs with cohort patients was performed. Repeated air samples for the same cohort of patients may be performed to monitor the change in viral load during hospitalization. Verbal consent was obtained from each patient or their parent. We collected the air samples using an AerosolSense Sampler (Thermo Fisher Scientific, MA) as previously described.¹⁷ Briefly, the air sample was collected through an omnidirectional inlet and was directed toward the collection substrate through an accelerating slit impactor at a flow rate of 200 L per minute for 6 hours. Air samples of 72,000 L were collected. The samples were sent to the microbiology laboratory within 30 minutes for further processing. The air sampler was placed at the corner of the AIIR at a distance >2 m from the patient's head (Fig. 1).

Viral load assessment of air and clinical samples

Upon receiving the air samples, the collection substrate was immersed in 2 mL VTM, and 1 mL medium was used for total nucleic acid extraction using the eMAG extraction system (bioMérieux, Marcy-l'Etoile, France) following the manufacturer's instructions. Quantifications of viral RNA or DNA in the air samples were performed using in-house real-time reverse transcription polymerase chain reaction (RT-PCR) as previously described.²⁰⁻²² The choice of in-house RT-PCR depended on the virological finding of clinical sample.

For the NPA specimens, total nucleic acid extraction was performed using 250 μ L of the specimen. RT-PCRs for specific respiratory viruses were performed for viral load assay as described above.

Epidemiological characteristics of patients with air dispersal of respiratory viruses

The demographics, clinical symptoms, presence of underlying diseases, and the medical treatment among patients with or without detectable viral genome by air samples were analyzed. The use of surgical mask by patients during air sample collection was recorded. A case-control analysis was performed to analyze the factors associated with air dispersal of respiratory viruses. Case and control were defined as patients with or without air dispersal of respiratory viruses, respectively.

Statistical analysis

The factors associated with air dispersal of respiratory viruses were analyzed using the Student t test or Fisher's exact test where appropriate. A 2-sided *P* value <.05 was considered statistically significant.

Results

Setting

Between December 3, 2021, and January 26, 2022, air sampling was conducted on 30 working days in the pediatric ward for 34 patients. Their NPA revealed parainfluenza virus 3 (PIF3) in 14 patients (41.1%), RSV in 9 patients (26.5%), human rhinovirus/enterovirus in 7 patients (20.6%), and adenovirus in 4 patients (11.8%). Of these 34 patients, 20 patients were singly isolated in AIIRs and another 14 patients shared double-bed AIIRs in which the patients were place near the air supply.

Epidemiological characteristics of patients with air dispersal of respiratory viruses

Of 20 singly isolated patients, 9 (45%) were male. The median age was 30 months (range, 3 months–15 years). Their NPA revealed PIF3 in 10 patients (50%), human rhinovirus/enterovirus in 5 patients (25%), RSV in 3 patients (15%), and adenovirus in 2 patients (10%). Rhinovirus-specific RT-PCR confirmed that

the 5 patients with human rhinovirus/enterovirus detection had rhinovirus in their NPA specimens. Of 20 patients, 7 (35%) had air dispersal of the same respiratory viruses (Table 1). None of these 20 patients wore a surgical mask during air sampling. In the case–control analysis, case patients had a significantly higher mean viral load in the NPA than the controls (Table 2). Of 7 patients with air dispersal of respiratory viruses, the mean viral load in the air samples was 1.58×10^3 copies/mL (range, $63-7.60\times 10^3$ copies/mL).

Of another 14 patients shared double-bed AIIRs, 6 (42.9%) were male. The median age was 15 months (range, 65 days–10 years). These 14 patients were grouped into 7 pairs with the same virological diagnosis in each double-bed AIIR (Table 3). Of these 14 patients, the mean viral load of respiratory viruses in their NPA was 4.64×10^7 copies/mL (range, 5.33×10^3 to 1.68×10^8 copies/mL). Except for a 10-year-old girl with RSV infection, all patients in the double-bed AIIRs did not wear surgical mask during air sampling. All air samples were positive, with a mean viral load of 1.02×10^4 copies/mL (range, $10-4.99 \times 10^4$ copies/mL). The mean viral load in air samples was significantly higher in AIIRs housing 2 patients than in AIIRs for singly isolated patients (1.02×10^4 copies/mL vs 1.58×10^3 copies/mL; P = .020).

Discussion

Air dispersal of respiratory viruses including PIF3, RSV, rhinovirus, and adenovirus were documented by the detection of viral load in the 72,000 L of air samples collected inside the AIIRs occupied by patients with symptomatic infections. In addition to the previous reports of airborne transmission of respiratory viruses,^{5,6} air dispersal of PIF3 was also recognized. Instead of collecting the exhaled air from the individual patients²³⁻²⁶ or performing the air sampling in the settings of emergency room or outpatient clinics with various confounding factors in the environment,^{27,28} this study is the first to demonstrate air dispersal in singly isolated patients with environmental control of air change, flow, temperature, and humidity in the AIIRs. Of 20 infected patients singly isolated in AIIRs, air dispersal was detected in 35%. The presence of air dispersal was only associated with the viral load in NPA but was not related to demographic characteristics, clinical symptoms, or use of bronchodilator and inhaled corticosteroids among the singly isolated patients. In addition, the mean viral load of respiratory viruses in room air sample was significantly higher in the AIIR caring for 2 patients with the same viral etiology than that in the AIIR caring for a single patient. This finding suggests that the burden of viral load among symptomatic infected cases was associated with the air dispersal of respiratory viruses.

The finding of air dispersal of respiratory viruses may have implications in infection prevention. Given the mean viral load in air samples of 1.58×10^3 copies/mL among the singly isolated patients, the total number of viral copies was 3.16×10^3 over a collection time of 6 hours because the collection substrate was immersed in 2 mL VTM. Assuming that the rate of air dispersal of respiratory viruses is static, 9 copies of viral genome were dispersed in the air per minute, which is comparable with the amount of air dispersal of SARS-CoV-2 RNA using the same air sampler in the same setting of AIIR.¹⁷ The infectious dose of respiratory viruses demonstrated in human volunteer studies by aerosol exposure varied from 0.68 median tissue culture infectious dose (TCID₅₀) for rhinovirus, to 0.5 TCID₅₀ for adenovirus, to 30-40 TCID₅₀ for RSV.²⁹ Using the correlation of 1 TCID₅₀ to 10^3 copies/mL,³⁰ we estimated the infectious doses Table 1. Epidemiological Characteristics of Patients Who Were Singly Isolated in Airborne Infection Isolation Room With Respiratory Tract Infection Associated With Detectable Viral Genome by Air Sampler

Patient Sex/a	Symptoms (Underlying Disease, ge If Any)	Respiratory Virus	Viral Load in NPA (Date of Collection)	Viral Load in Air (Date of Collection) ^a	Treatment	
1 M/3 y	Fever, cough, SOB (asthma)	PIF	2.87×10 ⁷ copy/mL (2 Dec 2021)	763 copy/mL (6 Dec 2021) ^b	Salbutamol puff, prednisolone, paracetamol, chlorphenamine	
2 F/3 y	Fever, cough, RN	PIF	9.26×10 ⁶ copy/mL (11 Dec 2021)	162 copy/mL (13 Dec 2021) ^b	Amoxicillin-clavulanate, paracetamol	
3 F/3 m	o Cough	PIF	9.76×10 ⁷ copy/mL (20 Dec 2021)	1,435 copy/mL (22 Dec 2021) ^b	Chlorphenamine	
4 M/31	no Fever, cough, SOB (cyclical neutropenia, asthma)	PIF	3.95×10 ⁷ copy/mL (26 Dec 2021)	63 copy/mL (28 Dec 2021) ^b	Salbutamol puff, prednisolone	
5 M/29	no Fever, cough, RN	RSV	1.64×10 ⁸ copy/mL (28 Dec 2021)	619 copy/mL (29 Dec 2021) ^b	Paracetamol	
6 F/20 r	no Fever, SOB	RSV	8.76×10 ⁷ copy/mL (9 Jan 2022)	382 copy/mL (17 Jan 2022) ^b	Salbutamol puff	
7 M/12	no Fever	Adenovirus	3.92×10 ⁵ copy/mL (7 Dec 2021)	7,602 copy/mL (8 Dec 2021) ^c	Paracetamol	

Note. NPA, nasopharyngeal aspirates; PIF, parainfluenza virus; RN, running nose; RSV, respiratory syncytial virus; SOB, shortness of breath. ^a72,000 L of air was collected over a 6-h period for each air sample. All patients did not wear surgical mask during air sample collection. During the viral load assay for air samples, the collection substrate was immersed in 2 mL of viral transport medium. Therefore, the viral load in air is expressed as the copy of viral genome per mL of viral transport medium. ^bDetectable viral RNA in air.

^cDetectable viral DNA in air.

Table 2. Case-Control Analysis of Patients With or Without Air Dispersal of Respiratory Viruses During Respiratory Tract Infection

Variable	Patients With Air Dispersal of Respiratory Viruses (n = 7), No. $(\%)^a$	Patients Without Air Dispersal of Respiratory Viruses (n = 13), No. (%) ^a	P Value
Age, mean mo ± SD	21±14	48±57	.312
Sex, male	4 (57.1)	5 (38.5)	.642
Respiratory viruses			
Parainfluenza virus 3	4 (57.1)	6 (46.2)	1
Respiratory syncytial virus	2 (28.6)	1 (7.7)	.270
Rhinovirus	0	5 (38.5)	.114
Adenovirus	1 (14.3)	1 (7.7)	.158
Symptoms			
Fever	6 (85.7)	5 (38.5)	.070
Cough	5 (71.4)	4 (30.8)	.160
Running nose	2 (28.6)	4 (30.8)	1
SOB	3 (42.9)	2 (15.4)	.290
Viral load of NPA			
Mean copy/mL	6.15×10 ⁷	1.61×10 ⁵	<.001
≥5 log ₁₀ ^b	7 (100)	5 (38.5)	.015
Day of air sampling after NPA collection (mean \pm SD) ^c	2.57±1.68	2.23 ± 1.93	.713
Use of medication			
Salbutamol	3 (42.9)	3 (23.1)	.613
Corticosteroid (inhaled or oral)	2 (28.6)	2 (15.4)	.587

Note. NPA, nasopharyngeal aspirates; SD, standard deviation; SOB, shortness of breath.

^aUnits unless otherwise indicated.

Wral load of NPA \geq 5 log₁₀ indicates high viral load in the clinical specimens. *Each patient had one air sample collection during hospitalization.

 Table 3. Epidemiological Characteristics of Patients Under Cohort Nursing in Airborne Infection Isolation Room With Respiratory Tract Infection Associated With

 Detectable Viral Genome by Air Sampler

Pair of Patients (Episode of Air Sampling)	Sex/Age	Symptoms (Underlying Disease, If Any)	Respiratory Virus	Viral Load in NPA (Date of Collection)	Viral Load in Air (Date of Collection) ^a	Treatment [Remark]
1 (1)	F/65 d	Cough (Down syndrome)	RSV	3.47×10 ⁷ copy/mL (23 Dec 2021)	10 copy/mL (30 Dec 2021) ^b	Salbutamol puff, paracetamol
1 (1)	F/10 y	Seizure (epilepsy)	RSV	4.10×10 ⁷ copy/mL (29 Dec 2021)	(00 200 2022)	Nil [wearing mask in AIIR]
2 (2)	F/5 mo	Fever, cough, RN	RSV	8.30×10 ⁵ copy/mL (6 Jan 2022)	5,745 copy/mL (11 Jan 2022) ^b	Salbutamol puff, amoxicillin- clavulanate
2 (2)	F/10 mo	Fever, cough, RN	RSV	1.97×10 ⁷ copy/mL (11 Jan 2022)		Amoxicillin-clavulanate
3 (3–5)	F/5 mo	Fever, cough, RN	RSV	5.42×10 ⁷ copy/mL (6 Jan 2022)	4.99×10 ⁵ copy/mL (12 Jan 2022) ^b ;	Salbutamol puff, amoxicillin- clavulanate
3 (3–5)	F/20 mo	Fever, SOB	RSV	8.76×10 ⁷ copy/mL (9 Jan 2022)	691 copy/mL (13 Jan 2022) ^b ; 474 copy/mL (14 Jan 2022) ^b	Salbutamol puff
4 (6–7)	M/6 y	Fever, cough (cerebral palsy)	PIF	1.68×10 ⁸ copy/mL (4 Jan 2022)	1.42 ×10 ⁵ copy/mL (4 Jan 2022) ^b ;	Salbutamol puff, paracetamol
4 (6–7)	M/24 mo	Fever (DD)	PIF	3.49×10 ⁷ copy/mL (3 Jan 2022)	9,188 copy/mL (5 Jan 2022) ^b	Paracetamol
5 (8)	F/4 mo	Cough	PIF	2.07×10 ⁴ copy/mL (20 Jan 2022)	1.27×10 ⁵ copy/mL (21 Jan 2022) ^b	Salbutamol puff
5 (8)	М/З у	Fever, cough	PIF	8.21×10 ⁷ copy/mL (20 Jan 2022)		Nil
6 (9)	M/18 mo	Cough, RN, SOB	Rhinovirus	6.17×10 ⁵ copy/mL (18 Jan 2022)	54 copy/mL (20 Jan 2022) ^b	Salbutamol puff, paracetamol
6 (9)	M/11 mo	Vomiting	Rhinovirus	3.38×10 ⁷ copy/mL (18 Jan 2022)		Nil
7 (10)	F/4 y	Fever, vomiting	Adenovirus	9.15×10 ⁷ copy/mL (24 Jan 2022)	5,150 copy/mL (25 Jan 2022) ^c	Paracetamol
7 (10)	M/9 mo	Cough, RN, SOB (CDH)	Adenovirus	5.33×10 ³ copy/mL (23 Jan 2022)		Salbutamol puff

Note. AllR, airborne infection isolation room; CDH, congenital heart disease; DD, developmental delay; NPA, nasopharyngeal aspirates; PIF, parainfluenza virus; RN, running nose; RSV, respiratory syncytial virus; SOB, shortness of breath.

^a72,000 L of air was collected over a 6-h period for each air sample. Except for a 10-year-old girl, all patients did not wear surgical masks during the air sample collection. During the viral load assay for air samples, the collection substrate was immersed in 2 mL viral transport medium. Therefore, the viral load in air is expressed as the copy of viral genome per mL of viral transport medium.

^bDetectable viral RNA in air.

^cDetectable viral DNA in air.

of rhinovirus (6.8×10² copies/mL), adenovirus (5.0×10² copies/ mL), and RSV $(3.0 \times 10^4 \text{ copies/mL})$. Considering the air sampling collection for 6 hours in AIIR, we translated the infectious dose in the room air in the AIIR to the number of viral copies for rhinovirus (1.36×10³ copies), adenovirus (1.0×10³ copies), and RSV $(6.0 \times 10^4 \text{ copies})$. Based on these findings, the maximum viral copies detected in 72,000 L of air in 6 hours in the AIIRs singly isolated for RSV was 1.24×10^3 copies, which may be lower than the infectious dose. However, our study was conducted in the AIIRs with 12 air changes per hour, which may have facilitated the dilution of infectious virus-laden particle in the air. Presumably, the viral copies in the air may be double in the general ward setting, which has 6 air changes per hour. Thus, outbreaks of respiratory viruses would be very common in general ward during the winter season when respiratory viruses are highly prevalent in patients with mild or no symptoms. Further investigation is needed to understand the degree of air dispersal of viral genome in the general ward setting with air ventilation of 6 air changes per hour, as well as in the community

setting with poor indoor air dilution, which is also a risk factor for SARS-CoV-2 transmission.^{31,32} Although the clinical significance of airborne transmission of respiratory viruses other than SARS-CoV-2 remains to be determined, the enforcement of infection control practice in the hospitals, including hand hygiene and universal masking, has successfully prevented nosocomial transmission of respiratory viruses and SARS-CoV-2 before the emergence of the omicron BA.2 variant.^{33,34}

This study had several limitations. We did not perform viral culture of the air samples. The demonstration of viral DNA or RNA may not correlate with the presence or level of viable virus. These sophisticated experiments have been performed in the investigation of airborne transmission of RSV.⁶ We could not include influenza A virus in this study because universal masking and enhancement of hand hygiene practice likely minimized the influenza activity in both community and hospital settings since the outbreak of COVID-19.^{34,35} The relative location of patients to the air sampler may have varied over the sampling time; some of our patients were pediatric cases who may have moved around

in the bed. The time lag from the collection of NPA to air samples may affect the correlation of viral loads between the clinical and air samples. In addition, our study was not adequately powered to measure all factors associated with air dispersal of respiratory viruses. However, given the small sample size, our findings clearly demonstrate that the viral load of the patient is an important factor. Further study to investigate the phenomenon of air dispersal of respiratory viruses is warranted.

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Conflict of interest. All authors report no conflicts of interest relevant to this article.

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