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11 **Frequent recovery of influenza A but not influenza B virus RNA in aerosols in pediatric**
12 **patient rooms**

13 Running head: Airborne influenza virus in pediatric wards

14

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65 **Abstract**
66 Influenza transmission occurs through the air, but the relative importance of small droplets,
67 or aerosols, in influenza transmission especially within healthcare facilities remains
68 uncertain. Detections of influenza virus in aerosols in cough and exhaled breath from
69 infected patients, and from the air in outpatient or inpatient healthcare facilities have been
70 studied, but most studies were done in adults with very few data involving children. We
71 aimed to assess the potential of influenza transmission via aerosols in pediatric patient
72 rooms. Two-stage cyclone (NIOSH) air samplers were used to collect the air in 5-bed
73 pediatric patient rooms with patients with PCR-confirmed influenza. Influenza A virus RNA
74 was recovered in 15/19 (79%) air sampling occasions, in all size fractions (>4μm, 1-4μm
75 and <1μm), and significantly less for influenza B virus (2/10 occasions, 20%). We estimated
76 a ventilation rate of 1.46 ACH in a similar but unoccupied 5-bed patient room. High
77 quantities of influenza A virus RNA detected in the air in pediatric patient rooms suggests
78 other individuals in paediatric patient rooms including other patients, visitors, caretakers
79 and healthcare workers could be exposed to influenza A virus while caring for infected
80 children.
81
82 **Keywords**: Influenza virus; influenza transmission; aerosol; pediatrics; healthcare settings;
83 infection control
84
85 **Practical implications:** Influenza virus has been thought to transmit predominantly via
86 droplet, but our study suggested the potential transmission via airborne route by detecting
87 substantial influenza A virus in the air. The viral detection in aerosols (<4μm) further
88 suggests a potential long-range transmission especially in a poorly ventilated setting.
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93 **BACKGROUND**

94 Influenza viruses are among the more important respiratory virus infections that cause
95 considerable morbidity and mortality in individuals of all ages every year.[1] Influenza viruses
96 are generally thought to transmit via multiple routes including contact, droplet and airborne
97 mode.[2] Contact transmission occurs when a patient with influenza (infector) directly
98 transfers virus-containing secretions to a susceptible person (infectee) such as shaking
99 hands (direct contact), or via a contaminated object or surface (indirect contact). Droplet
100 transmission occurs when the virus-containing large respiratory droplets from an infector
101 deposit onto the mucosal surfaces (eyes, nose, mouth) of an infectee. Airborne transmission
102 occurs when the virus-containing fine particle aerosols (usually believed to be particles with
103 aerodynamic diameter ≤5μm), generated during breathing, coughing or sneezing by the
104 infector,[3] is inhaled by an infectee and subsequently initiate the infection. Although many
105 have stated droplet transmission as the predominant mode in influenza transmission, the
106 relative importance of each transmission mode especially the fine particle aerosols remains
107 uncertain.[4] Infectious influenza virus was recovered in fine particles of <5μm from exhaled
108 breath of infected individuals.[5] Studies reported the detection of influenza virus in ambient
109 air in community[6] and healthcare settings.[6-10]

110

111 Children are thought to play a significant role in the transmission of influenza because of
112 less-developed immunity with increased susceptibility to infection, increased social contact
113 at schools, and possibly increased viral shedding for longer period of time,[11,12] but there are
114 only limited studies that investigated the importance of aerosol transmission in children.[13,14]
115 Tseng et al. recovered influenza A virus in 8/33 (24%) of the air samples collected in the
116 emergency room of a pediatrics department using a Nuclepore filter with 0.4μm pore size.[13]
117 Wan et al. recovered influenza A virus in 2/13 (15%) of air samples collected from a long
118 distance (3.2m) from the bed of influenza-infected pediatric patients in patient rooms using
119 a polytetrafluoroethylene filter with 0.2μm pore size, but none in air samples collected from
120 a short distance (0.6-1.8m) using a lower sampling airflow rate.[14]

121

122 There is an urgent need to evaluate the importance of aerosol transmission in children, in
123 particular in inpatient healthcare settings, because of the potential of increased

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susceptibility of children to influenza infection, the increased infectiousness of children with influenza, and increased transmission between caretakers and infected children than adults in inpatient settings. Here, we conducted an air sampling study with size fractionation in pediatrics patient rooms with patients with influenza-like illnesses in a tertiary hospital in China, and provided basic information on the ventilation in patient rooms. The objective of our study was to assess the potential of infected children transmitting influenza virus via aerosols by quantifying influenza virus RNA concentration in the air in pediatric patient rooms.

METHODS

Selection Criteria for Air Sampling

The First Affiliated Hospital of Guangzhou Medical University is a 1500-bed comprehensive 3A (tertiary) hospital in Guangzhou, China. The pediatric department houses 86 beds which are distributed in two floors. At hospital admission, throat swabs were routinely collected from pediatric patients and tested for respiratory virus infection either by reverse transcription polymerase chain reaction (PCR) or antigen test. To monitor for nosocomial infections, throat swabs were also taken and tested for respiratory virus infection if the patient developed respiratory symptoms during their hospital stay. For our study, we initiated air sampling in a patient room if at least one pediatric patient <14 years old who was present with fever plus one or more acute respiratory symptom (sore throat, cough, runny nose or fatigue) was identified. If ≥1 eligible patient was identified, the patient with highest influenza RNA copies was selected.

Air Sampling in Pediatric Patient Rooms

We sampled air in 5-bed pediatric infectious disease patient rooms (Figure 1). The distance between beds was within 1.1 to 1.9m. The patient rooms were disinfected with sodium troclosene reagent at least once per day. For air sample collection, we used a stationary set-up consisting of a two-stage cyclone sampler, developed by the US National Institute of Occupational Safety and Health (NIOSH),[15] mounted on a tripod at a height of 1.3m which is

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equivalent to the height of a child sitting on the bed. The NIOSH sampler collected air particles of >4μm in a 15mL centrifuge tube, particles of 1-4μm in a 1.5mL tube and particles of <1μm on a hydrophobic, polytetrafluoroethylene (PTFE) polymer membrane filter with 3.0μm pore size, 37mm in diameter (Merck Millipore, Germany). A portable analyzer that recorded temperature and relative humidity was also mounted on the tripod. In each sampling occasion, we placed two stationary set-ups in the selected patient room, with one placed near the head position (within 1m) of the selected patient, and the other sampler placed either near the head or the end of bed of a neighboring patient (approximately 2m from the selected patient). We collected air in the patient room for 4 hours continuously at a flow rate of 3.5L/minute. Other information including admission of new patients and opening of door/window were collected at 0, 2nd and 4th hours during the collection.

After each collection, 15mL tube (which collected air particles of >4μm) and 1.5mL tube (which collected air particles of 1-4 μm) were unscrewed from the sampler; while the PTFE filter (which collected air particles of <1μm) was removed from the rubber cassette and placed into a 15mL tube immediately. 1mL of virus transport media (consisted of minimum essential medium (MEM) with 0.5% gelatin, 0.05% bovine serum albumin (BSA), 20 μg/ml amphotericin B, 100 U/ml penicillin and 100 μg/ml streptomycin) was added into each of the three tubes. All tubes were then vortexed and spun down for 1 minute each. For the 15mL tube containing the PTFE filter, after spinning down the filter was removed from the tube. The virus transport media of all the three size-fractions of air samples were then aliquoted into 2ml tubes and stored in -80°C for subsequent laboratory analysis.

Estimating Ventilation in Patient Rooms

Both ventilation rate (i.e. the amount of outdoor air introduced into the room) and the total supply air flow rate into the room (i.e. the total amount of air, including recirculated air, supplied by the mechanical ventilation system) were measured in a different but identical 5-bed pediatric patient room without any patients in the same hospital. The ventilation rate was estimated using a tracer gas method.[16] The patient room was 86.32m^3 in size. Sulfur hexafluoride (SF6) was first injected into the cubicle with a constant emission rate of 150mL/minute controlled by a mass flow controller (MC 10SLPM, Alicat Scientific, USA). The

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185 SF6 concentrations at six different sampling points (above each of the five beds, and the
186 exhaust) inside the room were monitored continuously by a SF6 analyzer (KX-1000F,
187 Zhengzhou Kaixuan Tech Co., LTD, China) together with a multipoint sampler with flow rate
188 of 5L/minute. The injection of SF6 was stopped when steady state was reached, and the
189 decay of SF6 concentration was monitored until the SF6 concentration became very low
190 (approximately 2 hours). The ventilation rate (i.e. the air change rate) was then estimated
191 based on the SF6 concentration decay. The total supply air flow rate was estimated based on
192 the average air speed at the supply diffusers, measured using an anemometer (TESTO 435,
193 Lenzkirch, Germany), and the total supply air area.
194

195 **Laboratory Analysis**

196 RNA from air samples was extracted with 1mL of TRIzolTM reagent (Invitrogen Life
197 Technologies) and dissolved in RNase-free water. 300µl of air samples in virus transport
198 media were used for RNA extraction, and the final eluted purified RNA volume was 25µl.
199 Influenza virus RNA was identified by commercial TaqMan real-time PCR assay (Guangzhou
200 Institute of Respiratory Medicine Company Limited) according to the manufacturer's
201 protocols. In brief, 25µl of reaction mix containing Moloney murine leukemia virus reverse
202 transcriptase, Taq polymerase and 4µl of the RNA eluent were used for the real-time PCR.
203 Details of the PCR cycling conditions are as follows: an initial reverse transcription at 55°C
204 for 10 minutes, incubation at 94°C for 2 minutes, followed by 40 cycles of 94°C for 10
205 seconds and 55°C for 35 seconds (ABI-7500 real-time PCR instrument; Life Technologies,
206 Singapore). The limit of detection of the RT-PCR is 600 copies per 1ml original sample, and
207 we considered samples with clear reaction signal growth curve with Ct values ≤40 to be
208 positive for influenza.
209

210 In samples that were PCR-positive, virus culture with MDCK cells was done.[17] In brief, MDCK
211 cells were cultured to reach approximately 80-90% confluent and washed once with PBS
212 before inoculation with air samples. The suspension was removed after 1 hour of incubation,
213 and the cells were cultured in MEM containing 100 µg/ml streptomycin, 100 U/ml penicillin
214 and 2 µg/ml trypsin. The cells were then incubated for 2 to 3 days at 37°C with 5% CO_2. The
215 presence of cytopathic effects (CPE) was determined under a microscope.

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216

217 **Statistical Analysis**

218 We described the concentration of influenza A or B virus RNA detected per m^3 air in each 3
219 size fractions (>4μm, 1-4μm and <1μm) collected by each NIOSH air sampler; and number of
220 patients in the patient room positive for influenza by each air sampling occasion. We
221 compared the number of occasions with virus RNA detected in the air between influenza A
222 and B virus in all air sampling occasions, or only in occasions with influenza-positive
223 patients by Fisher's exact test. In the subset of air sampling occasions with only one
224 influenza A-infected patient, for each air size fraction we investigated any significant
225 difference in viral load between the two air samplers placed at different locations by
226 Wilcoxon signed-rank test. All analyses were conducted with R version 3.5.1 (R Foundation
227 for Statistical Computing, Vienna, Austria).

228

229 **Ethics Statement**

230 This study was approved by the Institutional Review Boards of The University of Hong Kong
231 and The First Affiliated Hospital of Guangzhou Medical University. All parents and legal
232 guardians provided oral informed consent. Written consent was deemed unnecessary
233 because the study involved only environmental sampling and information related to patient
234 diagnoses was collected anonymously.

235

236 **RESULTS**

237 **Characteristics of the Patients and Sampling Occasions**

238 During the local influenza seasons in three consecutive years (2015-17), we performed 26
239 air sampling occasions in 5-bed pediatric patient rooms, contributing to 156 air samples
240 from all size fractions. Pediatric patients presented during the air sampling occasions had a
241 mean age of 3.5 years old (IQR 0.6-4.8), with average body temperature of 37.4°C (IQR 36.8–
242 38.2) measured at admission and hospital stay of 8 days (IQR 5–10). Common respiratory
243 diagnoses were pneumonia (72%) and upper respiratory infections (34%); other non-
244 respiratory diseases included tonsillitis (5%) and enteritis (7%). Room temperature and
245 relative humidity was on average 24°C (IQR 23–25) and 73% (IQR 67–77). We recorded the

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number of HCWs and visitors during the 3 sampling time-points: at least 3 visitors and at most 4 HCWs were present during any sampling episode.

Influenza Virus RNA Detection in the Air

During all 26 air sampling occasions conducted, there was on average 0.9 (SD 0.7) patients with laboratory-confirmed influenza A and 0.6 (SD 0.9) patients with influenza B virus infections. We recovered influenza A or B virus RNA in 22/26 (85%) and 2/26 occasions (8%) respectively. In particular, 19/26 (73%) occasions had ≥1 patient (mean 1.3 patients, SD 0.6) with laboratory-confirmed influenza A virus infections, and 10/26 (38%) occasions had ≥1 patient (mean 1.5 patients, SD 0.7) with laboratory-confirmed influenza B virus infections. From these, we recovered influenza A virus RNA in 15/19 (79%) (Table 1) and influenza B virus RNA in 2/10 (20%) occasions (Table 2) respectively. We also recovered influenza A virus in the air from the 7 occasions with no patients found to have laboratory-confirmed influenza A virus infection (Table 1), but none from the 16 occasions where no patient was infected with influenza B virus (Table 2). Probability of detection in the air was significantly higher for influenza A than B virus, whether in all 26 sampling occasions ($p=2.31\text{x}10^{-8}$), or only in occasions with ≥1 patient positive for the corresponding influenza A or B virus infections ($p=4.52\text{x}10^{-3}$).

Due to limited number of air samples with influenza B virus detected, further analyses focused on influenza A virus only. In all the 26 sampling occasions, influenza A virus RNA was frequently detected in all air size fractions <1μm (13/26, 50%), 1-4μm (11/26, 42%) and >4μm (16/26, 62%) (Figure 2). Virus culture was done for almost all air samples with influenza A virus RNA detected by PCR but none were culture-positive. In 10 air sampling occasions in which only the selected patient had laboratory-confirmed influenza A infection, we recovered influenza A virus in the air from both air samplers that were placed near (within 1m) the selected patient (7/10 sampling episodes), or next to a neighboring patient (6/10 sampling episodes) approximately 2m apart from the selected patient. Influenza A virus was detected more frequently near the selected patient (5/10 in <1μm, 4/10 in 1-4μm and 6/10 in >4μm) than the neighboring patient (3/10 in <1μm, 3/10 in 1-4μm and 2/10 in

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>4 μm). However, no significant difference in viral load was observed between the two air samplers for air size fractions <1μm (p=0.27), 1-4μm (p=0.82), and >4μm (p=0.39).

Ventilation of the Patient Rooms

The steady state SF6 concentrations at the six sampling points all fell within the range of 100 to 120 ppm. Based on the SF6 concentrations decay, we estimated the ventilation rate was 1.46 ACH. Separately, the measured average air speed at the supply diffusers was 2.15 m/s and the total supply air area was 0.14 m^2. Since the volume of the patient room was 86.32 m^3, we estimated the total supply airflow rate was 12.24 ACH.

DISCUSSION

Influenza virus infections in children are occasionally severe enough to warrant hospitalization. Within healthcare facilities, nosocomial infection may occur if infected patients and susceptible individuals occupy the same area. The present study is among the few to evaluate influenza aerosols in healthcare settings particularly from children.[18] We were able to identify influenza A (Table 1), and to a significantly lesser extent influent B virus (Table 2), in air particles including both droplets (>4μm) and aerosols (1-4μm and <1μm fractions) (Figure 2) collected from pediatric patient rooms, consistent with the estimates from a study of secondary transmission in influenza-exposed household contacts.[19] Lindsley et al. also showed more detection of airborne influenza A than B virus RNA in an urgent care medical clinic, although it was attributed to a higher prevalence of influenza A virus during the study period and no patient with influenza B virus infection was identified.[10] Moreover, when there was a patient with influenza A in the ward, we had a 79% chance of detecting influenza A virus RNA in the air, compared to just a 20% chance of detecting influenza B virus RNA when there was an influenza B patient in the ward.

Our study was conducted in a natural hospital setting. Our study did not aim to observe transmission directly as it is difficult to attribute a transmission event solely to a particular route of transmission; however, the high prevalence of influenza A virus RNA in the air in patient rooms implies other patients, health care workers and visitor could be exposed to

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306 potential infection via contaminated air. On the other hand, although we detected influenza
307 A virus RNA in the air in most occasions, none of them were culture positive. In comparison
308 to one study which isolated viable avian influenza virus from air samples with over 10^5 RNA
309 copies/m^3 air using the same NIOSH samplers,[20] we expected a low probability of recovering
310 viable virus from aerosols in our study. Cao et al. evaluated the collection efficiency of
311 NIOSH sampler and reported a very low retention of virus infectivity with significant decline
312 after 60 minutes of collection, and suggested the loss of infectivity due to desiccation or
313 degradation.[21] Two other studies evaluated the collection efficiency of other commercially
314 available air samplers and similarly inferred a loss of infectivity in air samples due to drying
315 of the aerosol particles.[22,23] A previous study showed that if an enhanced infectivity
316 detection method is used infectious viruses could be identified from air samples with 10^7
317 viral copies/m^3 air collected using the NIOSH samplers.[21] Despite the inability to recover
318 infectious virus in our air samples, if for aerosol transmission the putative human infectious
319 dose (HID) was 0.6–3 $TCID_{50}$ and equivalent to 90–1950 RNA copies,[4,8,24] all our PCR-
320 positive samples exceeded the upper bound of the HID_{50} and might indicate the potential to
321 initiate infection via the aerosol route.
322
323 Systematic measures such as increasing ventilation rates of patient rooms might be more
324 feasible than providing and ensuring personal respiratory protection of healthcare workers,
325 visitors or nearby patients. It is suggested that ventilation may play a role in reducing the
326 risk of influenza transmission,[25] and therefore could be an especially important engineering
327 intervention in healthcare settings if the aerosol route is found to be important for the
328 nosocomial transmission of influenza. In the present study, it was not possible to measure
329 the ventilation in parallel with each air sampling session as there were occupants in the
330 room. Instead, we estimated the ventilation rate and the total supply air flow rate in a similar
331 5-patient room within the same hospital using data obtained from an earlier study. The
332 steady state SF6 concentrations at all the sampling points were found to be very close,
333 together with the high total supply air flow rate, indicated the air was relatively well mixed
334 in the patient room. Although the estimated ventilation rate of 1.46 ACH was slightly less
335 than the suggested value of 2 ACH for patient rooms by the Chinese national guidelines,[26] we
336 would expect a similar influenza virus detection rate in the air would be observed even if the

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337 suggested ventilation rate was reached. Therefore, our results suggested that further
338 research is needed to design a ventilating system that could minimize the transmission of
339 nosocomial infections through the aerosol route but at the same time cost-effective, for
340 diseases which we expect to be less severe. Design of ventilation systems that minimize
341 airflow from one patient to another patient or to the surrounding should also be considered
342 since direction of airflow has been suggested to associate with the spread of airborne
343 infectious diseases.[27,28]
344
345 We recovered about 1 $\log_{10}$ higher viral load in the present study conducted in 5-bed
346 pediatric patient rooms in Guangzhou (Figure 2), when compared to our earlier similar
347 study in 2-bed adult patient rooms in Hong Kong.[7] Such difference might be explained by
348 differences in environmental factors (e.g. ventilation, temperature and relative humidity), or
349 the number and infectiousness of infected individuals in the patient rooms. For the adult
350 patient rooms in our previous study, a negative pressure isolation ward, the ventilation rate
351 was maintained at 12 ACH.[7] There were more visitors and infected patients in the pediatric
352 than the adult patient rooms which could possibly contribute to a higher viral load in the
353 pediatric environment. Some studies postulated children may be more infectious than adults
354 by longer duration of virus shedding[18] or increased peak viral load, but a systematic review
355 found no difference in quantity of virus RNA in respiratory swabs by age.[29]
356
357 Our study has several limitations. First, we could not identify any viable influenza A virus by
358 virus culture from PCR-positive air samples as discussed above. Second, we were not able to
359 estimate the ventilation rate in parallel for each session of the air sample collection, for
360 example by tracer gas method as the patient rooms were in use, nor by indoor carbon
361 dioxide concentration increment above the outdoor level as there were frequent changes in
362 the numbers of individuals in the patient rooms.[30] Instead, we provided information on the
363 ventilation estimated in a similar 5-patient room within the same hospital using data
364 obtained from an earlier study for indicative purposes. Third, we were not able to confirm
365 the source of virus generation as identified in the air samples. Some patients were diagnosed
366 with antigen test and therefore lacked the data on viral shedding. Although more virus
367 detection of influenza virus in the air collected from infected patient than the neighboring

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368 (uninfected) patient suggested patients with laboratory-confirmed infection in the sampled
369 patient rooms were the likely source of the virus in the air, we also detected influenza virus
370 in rooms in the absence of laboratory-confirmed cases. Other individuals in the room
371 including patients without respiratory symptoms, visitors and healthcare workers, or
372 inadequate ventilation in the hospital, might have contributed to viruses in the air in these
373 occasions. In future studies, virus sequencing might be used to link the viruses detected in
374 the air with individual patients.

375

376 As seasonal influenza virus is thought to transmit predominantly via droplets, currently
377 Droplet Precautions is recommended for healthcare workers when caring for influenza
378 patients.[31,32] Droplet Precaution measures include proper use of personal protective
379 equipment for example surgical masks, appropriate patient placement for example either in
380 single rooms or with other patients infected by the same pathogen, and reducing patient
381 movement.[31,32] Because influenza A virus RNA was identified frequently in the air in
382 paediatrics patient rooms in the present study, some may raise the concerns on the need to
383 adopt Airborne Precautions, which would entail the use of respirators and isolation of
384 patients in negative-pressure airborne isolation infection rooms (AIIR). Although our
385 present findings and other similar studies[33] demonstrated the presence of airborne
386 influenza virus RNA, evidences on the infectivity of these airborne virus remains very
387 limited. As we discussed in a recent review on the controversy of airborne transmission of
388 respiratory viruses and the implications for infection prevention in healthcare settings,
389 additional studies to identify the presence of viable (infectious) virus in the recovered air
390 samples, and infection in susceptible individuals initiated from the inhalation of airborne
391 viruses, are needed to provide more definitive support on the importance of aerosol
392 transmission for influenza. Furthermore, Airborne Precautions for a respiratory disease will
393 only be justified if the disease is believed to be with moderate or high severity.[34]

394

395 In conclusion, our findings suggested there is a greater potential of aerosol transmission of
396 influenza A and less for influenza B virus; and other individuals in paediatrics patient rooms
397 including other patients, visitors, caretakers and healthcare workers could be exposed to
398 influenza A virus aerosols while caring for infected children.

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Table

Table 1. Recovery of influenza A virus RNA in the air from 5-bed pediatric patient rooms over a 4-hour sampling period.

Two NIOSH samplers mounted on separate tripods were used in each sampling occasion. One NIOSH sampler (NIOSH 1) was placed near the head position of the bed of the selected patient, and the other NIOSH sampler (NIOSH 2) near the head or the end of the bed of a neighboring patient. The two NIOSH samplers were placed approximately 2 meters apart. We attempted to recover viable virus from all PCR-positive samples by culture (except for occasions 1, 2, 12 and 18 where there were insufficient samples) but all were culture negative.

Air sampling occasions are numbered in chronological order. '-' represents viral RNA not detected in the air sample by PCR. * indicates if the selected or neigboring patient had laboratory-confirmed influenza A virus infection. Ventilation conditions, i.e. opening of door or window, were recorded as opened ('Y'), closed ('N') or changed ('Mixed') during the course of air sampling. N/A: Not applicable.

Table 2. Recovery of influenza B virus RNA in the air from 5-bed pediatric patient rooms over a 4-hour sampling period.

Two NIOSH samplers mounted on separate tripods were used in each sampling occasion. One NIOSH sampler (NIOSH 1) was placed near the head position of the bed of the selected patient, and the other NIOSH sampler (NIOSH 2) near the head or the end of the bed of a neighboring patient. The two NIOSH samplers were placed approximately 2 meters apart. We attempted to recover viable virus from all PCR-positive samples by culture (except for occasions 1, 2, 12 and 18 where there were insufficient samples) but all were culture negative.

Air sampling occasions are numbered in chronological order. '-' represents viral RNA not detected in the air sample by PCR. * indicates if the selected or neigboring patient had laboratory-confirmed influenza B virus infection. Ventilation conditions, i.e. opening of door or window, were recorded as opened ('Y'), closed ('N') or changed ('Mixed') during the course of air sampling. N/A: Not applicable.

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Table 1. Recovery of influenza A virus RNA in the air from 5-bed pediatric patient rooms over a 4-hour sampling period.

Air sampling occasion	Month/Year	Influenza A virus viral load (copies/m^3 air)								Patient information					Ventilation condition	
		Air particles from NIOSH sampler at selected patient				Air particles from NIOSH sampler at neigboring patient										
		<1µm	1-4µm	>4µm	Total	<1µm	1-4µm	>4µm	Total	No. positive patients / No. tested	Flu A patient bed No.	No. Flu A patients used nebulizer	Selected patient bed no.	Neighboring patient bed no.	Door opened	Window opened
With at least 1 patient with laboratory-confirmed influenza A infection (n = 19)																
1	06/15	-	-	-	-	-	-	-	-	2/4	2,4	0	4*	5	Y	N
2	06/15	-	-	-	-	-	-	-	-	1/2	4	0	4*	5	Y	N
3	06/15	-	-	-	-	-	-	-	-	1/3	4	0	4*	5	Mixed	N
4	06/15	-	-	-	-	-	-	18993	18993	1/3	2	0	2*	1	Y	N
5	07/15	31583	28812	10274	70669	19676	38762	19815	78253	3/4	1,2,4	2	2*	1*	Y	Y
6	07/15	14729	19263	14117	48109	-	-	-	-	1/3	3	0	3*	2	Y	Mixed
7	07/15	-	-	35361	35361	-	-	-	-	1/4	3	0	2	1	Y	N
10	07/15	32259	39873	47239	119371	-	57569	13724	71293	1/5	5	0	5*	4	Y	N
12	04/16	17205	-	-	17205	-	-	-	-	1/5	1	0	4	5	Y	N
13	04/16	-	14117	-	14117	18464	-	-	18464	1/4	3	1	1	2	Y	N
14	04/16	24492	8253	-	32745	-	9709	15919	25628	1/4	5	1	3	2	Y	N
15	04/16	-	3715	-	3715	12001	-	-	12001	1/3	5	0	5*	4	Y	N
16	04/16	-	-	-	-	-	-	-	-	2/5	1,3	1	5	4	Y	N
19	05/16	-	-	7319	7319	-	25016	-	25016	1/4	1	0	2	1*	Y	N
20	05/16	-	-	20097	20097	-	-	-	-	1/6	2	0	2*	1	Y	N
21	05/16	3487	-	8733	12220	13342	-	-	13342	1/5	4	0	4*	5	Y	Y
23	05/16	-	31140	-	31140	-	-	-	-	2/4	2,4	1	4*	5	Y	N
25	07/17	27616	16608	-	44224	16259	16375	34134	66768	1/4	3	0	3*	2	Mixed	Mixed
26	08/17	45923	-	25372	71295	-	23978	-	23978	1/4	5	0	5*	4	Y	N
Without any patient with laboratory-confirmed influenza A infection (n = 7)																
8	07/15	-	-	-	-	-	-	20383	20383	0/5	N/A	0	2	1	Y	N
9	07/15	50339	-	16259	66598	18334	-	-	18334	0/4	N/A	0	4	5	Mixed	Mixed
11	04/16	-	-	-	-	-	-	26658	26658	0/3	N/A	0	4	5	Y	N
17	04/16	-	-	-	-	-	-	16844	16844	0/5	N/A	0	4	5	Y	N
18	04/16	-	-	-	-	10948	-	-	10948	0/5	N/A	0	3	2	Mixed	N
22	05/16	28408	-	11916	40324	284078	-	-	284078	0/5	N/A	0	1	2	Y	N
24	07/17	19676	21568	-	41244	-	16031	-	16031	0/5	N/A	0	3	2	Y	Y

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Table 2. Recovery of influenza B virus RNA in the air from 5-bed pediatric patient rooms over a 4-hour sampling period.

Air sampling occasion	Month/Year	Influenza B virus viral load (copies/m³ air): Air particles from NIOSH sampler at selected patient				Influenza B virus viral load (copies/m³ air): Air particles from NIOSH sampler at neigboring patient				Patient information					Ventilation condition	
		<1μm	1-4μm	>4μm	Total	<1μm	1-4μm	>4μm	Total	No. positive patients / No. tested	Flu B patient bed no.	No. Flu B patients used nebulizer	Selected patient bed no.	Neighboring patient bed no.	Door opened	Window opened
With at least 1 patient with laboratory-confirmed influenza B infection (n = 10)																
11	04/16	-	-	-	-	-	-	-	-	3/3	2,3,4	1	4*	5	Y	N
12	04/16	-	-	-	-	2801	-	-	2801	1/5	4	0	4*	5	Y	N
14	04/16	-	-	-	-	-	-	-	-	2/4	1,3	0	3*	2	Y	N
15	04/16	-	9176	-	9176	-	-	-	-	2/3	1,3	0	5	4	Y	N
16	04/16	-	-	-	-	-	-	-	-	1/5	5	0	5*	4	Y	N
17	04/16	-	-	-	-	-	-	-	-	2/5	2,4	0	4*	5	Y	N
18	04/16	-	-	-	-	-	-	-	-	1/5	3	0	3*	2	Mixed	N
19	05/16	-	-	-	-	-	-	-	-	1/4	1	0	2	1*	Y	N
21	05/16	-	-	-	-	-	-	-	-	1/5	3	0	4	5	Y	Y
22	05/16	-	-	-	-	-	-	-	-	1/5	1	0	1*	2	Y	N
Without any patient with laboratory-confirmed influenza B infection (n = 16)																
1	06/15	-	-	-	-	-	-	-	-	0/4	N/A	0	4	5	Y	N
2	06/15	-	-	-	-	-	-	-	-	0/2	N/A	0	4	5	Y	N
3	06/15	-	-	-	-	-	-	-	-	0/3	N/A	0	4	5	Mixed	N
4	06/15	-	-	-	-	-	-	-	-	0/3	N/A	0	2	1	Y	N
5	17/15	-	-	-	-	-	-	-	-	0/4	N/A	0	2	1	Y	Y
6	07/15	-	-	-	-	-	-	-	-	0/3	N/A	0	3	2	Y	Mixed
7	07/15	-	-	-	-	-	-	-	-	0/4	N/A	0	2	1	Y	N
8	07/15	-	-	-	-	-	-	-	-	0/5	N/A	0	2	1	Y	N
9	07/15	-	-	-	-	-	-	-	-	0/4	N/A	0	4	5	Mixed	Mixed
10	07/15	-	-	-	-	-	-	-	-	0/5	N/A	0	5	4	Y	N
13	04/16	-	-	-	-	-	-	-	-	0/4	N/A	0	1	2	Y	N
20	05/16	-	-	-	-	-	-	-	-	0/6	N/A	0	2	1	Y	N
23	05/16	-	-	-	-	-	-	-	-	0/4	N/A	0	4	5	Y	N
24	07/17	-	-	-	-	-	-	-	-	0/5	N/A	0	3	2	Y	Y
25	07/17	-	-	-	-	-	-	-	-	0/4	N/A	0	3	2	Mixed	Mixed
26	08/17	-	-	-	-	-	-	-	-	0/4	N/A	0	5	4	Y	N

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Figure

Figure 1. Layout of 5-bed patient room where the air sampling was conducted. The distance between beds was 1.7–1.9m on the side with the restroom, and 1.1 – 1.7m on the opposite side. During each air sampling occasion, there were two NIOSH sampler-set up (i.e. one NIOSH sampler mounted on a tripod connected to a pump which was stored inside a sound-proof box), one placed near the head position (within 1m) of the selected patient (NIOSH 1) and the other set-up placed near the head or the end of bed of the neighboring patient (NIOSH 2). The two NIOSH samplers were placed approximately 2 meters apart. This figure is given as an illustration of the positions of the NIOSH air samplers relative to the selected patient, where the selected patient could in reality be on any beds (number 1 – 5).

Figure 2. Distribution of influenza A virus RNA recovered in different size-fractions of air particles. Influenza A virus RNA in the air was detected from both NIOSH samplers in all size-fractions of air particles. Samples negative, is below the lower limit of detection of the PCR assay, are plotted as 'Undetectable'. Limit of detection (LOD) is defined as 600 copies per 1ml original sample, which converts to 714 copies per m^3 in our samples.

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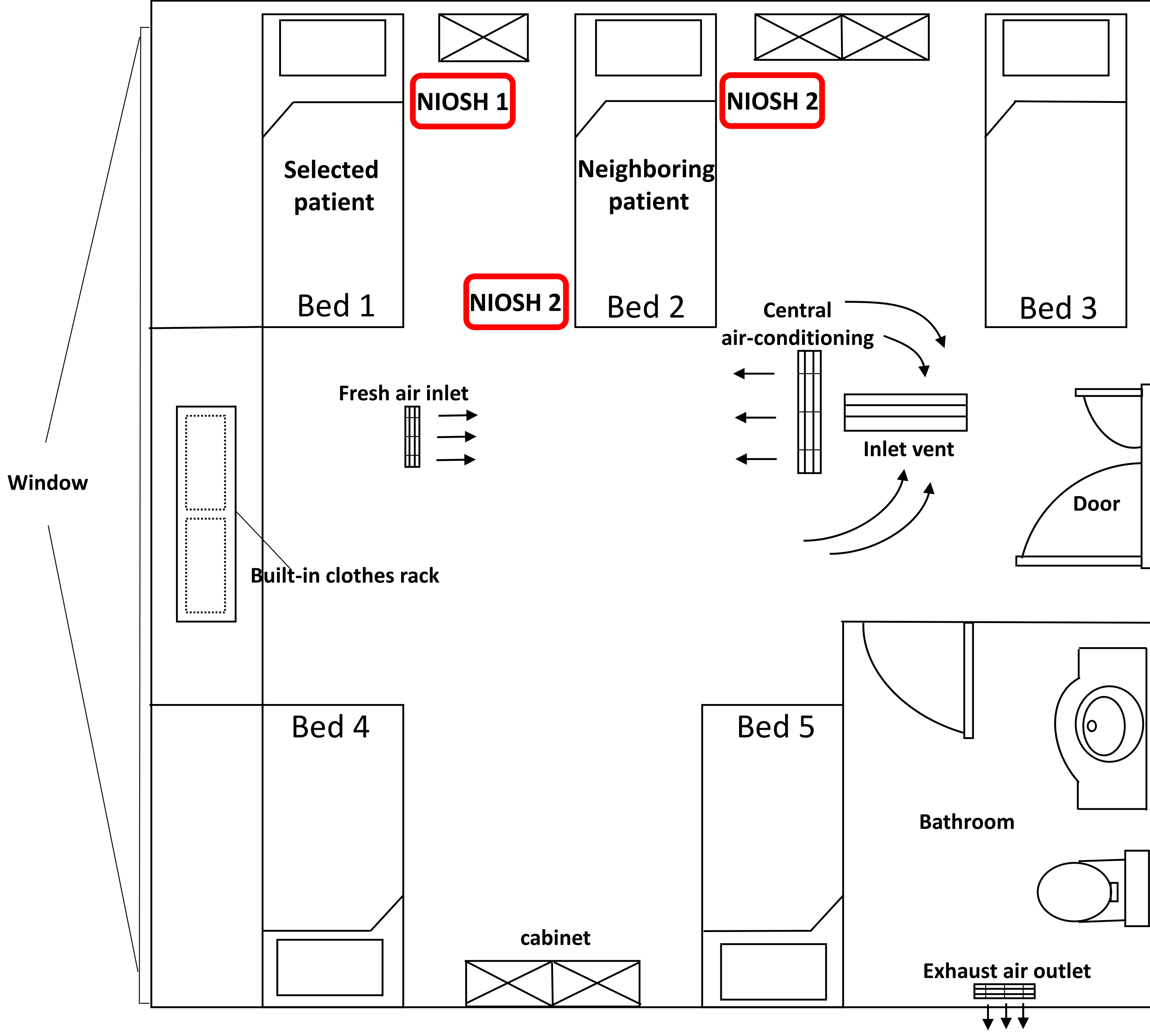
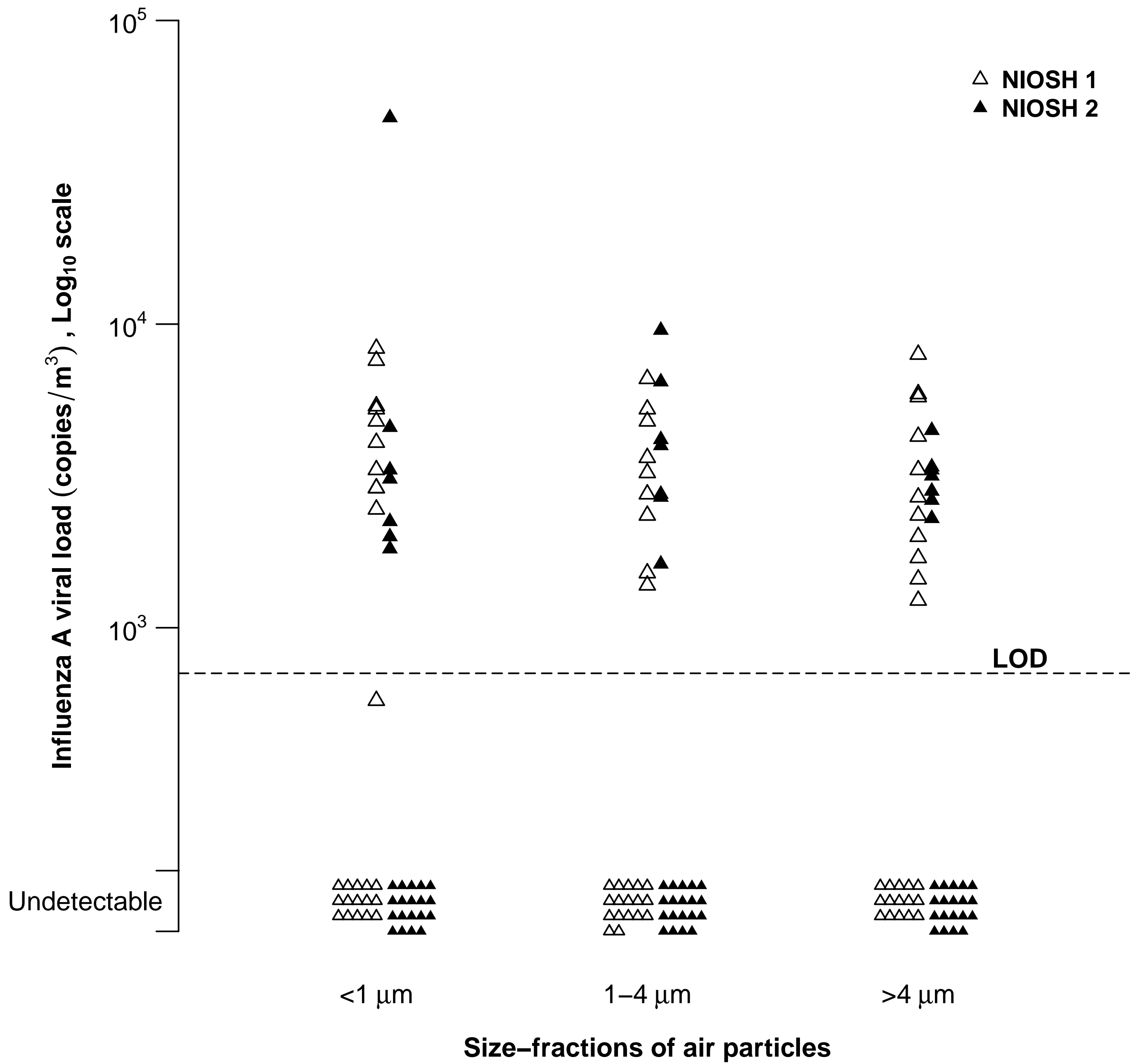

NIOSH 1
NIOSH 2
Selected patient
Neighboring patient
Bed 1
NIOSH 2
Bed 2
Central air-conditioning
Bed 3
Fresh air inlet
Inlet vent
Window
Door
Built-in clothes rack
Bed 4
Bed 5
Bathroom
cabinet
Exhaust air outlet



NIOSH 1
NIOSH 2
Influenza A viral load (copies/m3) , Log10 scale
10^5
10^4
10^3
LOD
Undetectable
<1 µm
1–4 µm
>4 µm
Size–fractions of air particles