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Airborne transmission of SARS-CoV-2: what is the implication of hospital infection control?

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To the Editor – Airborne transmission of severe acute respiratory syndrome associated coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has been increasingly recognized in the indoor air environment,[1] especially in the poorly ventilated premises.[2] In the recent update of scientific brief by Centers for Disease Control and Prevention, the modes of SARS-CoV-2 transmission includes inhalation of very fine respiratory droplets and aerosol particles, in addition to deposition of virus on exposed mucous membranes, and touching mucous membranes with soiled hands contaminated with virus.[3] Nosocomial outbreak of COVID-19 was possibly attributed to airborne transmission in an old-fashioned general ward with low ceiling height, despite 6 air change per hour (ACH).[4] To establish the role of airborne transmission of SARS-CoV-2 in the healthcare setting, it is important to demonstrate the presence of SARS-CoV-2 RNA and preferably viable virus in the air sample. However, it is a challenging experiment. In the previous reports of air sampling in the clinical areas, the findings were inconsistent.[5] It is not unexpected because air samplers with different mechanisms of sample collection such as solid impactors, liquid impactors, filters, and other sampling methods, were used. In addition, the testing protocols were different in terms of the relative position between patients and air samplers, number of patient in the room or ward, volume of air collection per sample, and the ACH of the patient care areas. The patient factors included the severity of clinical symptoms, the presence of aerosol-generating procedure (AGP), viral load of clinical specimens, and with or without wearing surgical mask during sample collection. Nevertheless, detection of SARS-CoV-2 RNA in air in the healthcare setting is summarized in the Supplementary Table. Most of these studies did not mention the patients' viral load and the use of surgical mask during sample collection.

To demonstrate the SARS-CoV-2 RNA viral load correlation between air and clinical samples, we performed air sampling in the airborne infection isolation room (AIIR) (16 square meters and 12 ACH), singly cared for an asymptomatic COVID-19 patient, who was an imported case with SARS-CoV-2 PANGO lineage B.1.525 from June 11, to June 17, 2021. No AGP was performed during air sample collection. We collected the air sample using a recently launched AerosolSense Sampler (Thermo Fisher Scientific, MA),[6] which is ~14 inches in three dimensions and 26 Ibs in weight, and placed 1 meter adjacent to patient's head. A single-use sampling cartridge containing 1-inch collection substrates is installed into the sampler. The air sample is

collected through an omnidirectional inlet and directed toward the collection substrate through an accelerating slit impactor at a flow rate of 200L per minute. Particles are trapped on the collection substrate as the air moves around the collection area. After the sampling cycle of 2, 4 and 8 hours in patient with or without wearing surgical mask, the sample cartridge is removed and sent to microbiology laboratory within 30 minutes. The collection substrate is then immersed into 1.5 ml viral transport medium, and 250 μl of medium were used for total nucleic acid extraction using the eMAG extraction system (bioMérieux, Marcy-l'Etoile, France) following manufacturer's instruction. Quantifications of SARS-CoV-2 RNA in the air samples were performed by the ultra-sensitive reverse-transcriptase droplet digital polymerase chain reaction (RT-ddPCR) using the QX200 Droplet Digital PCR System (Bio-Rad Life Science, CA, USA) as previously described.[7] The nasopharyngeal swabs were subjected to the same laboratory processing protocol. The viral loads of the air and clinical samples were summarized in Table 1.

Our finding has implication in hospital infection control. In contrast to our previous report of undetectable SARS-CoV-2 RNA in collecting 1,000L air,[8] the SARS-CoV-2 RNA was detected at a concentration of 0.009 copies/L in a COVID-19 patient, without wearing surgical mask, with moderate level of viral load (6,828,801 copies/ml) in nasopharyngeal swab when 96,000 L air was collected in 8 hours. SARS-CoV-2 RNA was also detected (0.005 copies/L) in another 8-hour air sample despite wearing surgical mask. It appears that a low quantity of SARS-CoV-2 RNA can be detected in air even in the AIIR with 12 ACH, when a large volume of air is collected for a prolonged period. If the experiment is performed in a general ward with 6 ACH, a higher quantity of SARS-CoV-2 RNA may be detected in air. Then, inhalation of SARS-CoV-2 by patient may be possible if there is an unrecognized COVID-19 case in the same cubicle.[4] In that case, portable high-efficiency particulate filter may be installed, especially in the old-fashioned ward with suboptimal ventilation. In addition to the recommendation of wearing surgical respirator among healthcare workers (HCWs) during AGP, we also suggest universal masking of patient and HCWs workers to reduce the risk of nosocomial transmission SARS-CoV-2 in the healthcare setting,[9] when the herd immunity of COVID-19 vaccination is not achieved.

Similar to the healthcare system in other developed areas, we manage COVID-19 patients in hospital AIIR with 12 ACH or community treatment facility (CTF) with air ventilation of

80L/s/person in Hong Kong.[10] Full personal protective equipment including surgical respirator, cap, face shield, gown, and gloves is mandated when caring for COVID-19 patients. The risk of inhalation of SARS-CoV-2 by HCWs in the hospital AIIR or CTF is extremely low. However, transmission of SARS-CoV-2 is not only limited to airborne route. Infection control professional should update HCWs workers with new scientific evidence while enforcing hand hygiene, standard, contact, and droplets precautions to prevent nosocomial outbreak of COVID-19.

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Conflict of interest.

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

Table 1. SARS-CoV-2 RNA viral load correlation between clinical and air samples in airborne infection isolation room singly cared for an asymptomatic COVID-19 patient[a]

Sampling no. [b]	Wearing surgical mask during air sampling [c]	Duration / total volume of air collection [d]	VL of NPS (copies per ml) [e, f]	VL of air sample (copies per sample) [f]	VL of air sample (copies per L of air)
1	No	2 hours / 24,000 L	355,692	ND	-
2	Yes	2 hours / 24,000 L	355,692	ND	-
3	No	4 hours / 48,000 L	14,140	ND	-
4	Yes	4 hours / 48,000 L	14,140	ND	-
5	No	8 hours / 96,000 L	6,828,801	774	0.009
6	Yes	8 hours / 96,000 L	974	497	0.005

Note. ND, not detected; NPS, nasopharyngeal swab; SARS-CoV-2, severe acute respiratory syndrome associated coronavirus 2; VL, viral load.

[a] No aerosol-generating procedure was performed during the air sample collection.

[b] The air samples were collected in day time. To explore the presence of SARS-CoV-2 RNA in the air, the first sample (sample 5) was collected for 8 hours in the airborne infection isolation room where patient not wearing surgical mask. Subsequently, we collected air samples at 2-hour and 4-hour interval in the same patient with or without wearing surgical mask, and followed by the last air sample (sample 6, 8-hour with wearing surgical mask).

[c] The COVID-19 patient wore American Society of Testing and Materials level 1 standard surgical masks during air sampling.

[d] AerosolSense Sampler (Thermo Fisher Scientific Inc., MA) is used. The airflow rate is 200 L per minute.

[e] The viral load of NPS collected on the same day of air sampling.

[f] Specific primer/probe set targeting the SARS-CoV-2 N2 gene and the human housekeeping gene RNase P gene were assessed for use in RT-ddPCR. The cycling conditions were: 50°C (60 min), 95°C (10 min), 40 cycles of 94°C (30 sec) and 60°C (1 min), 98°C (10 min), 4°C (30 min), 4°C (∞). Data analysis was performed by using the QuantaSoft Analysis Pro Sofware (Bio-Rad Life Science, CA, USA).

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Supplementary Table. Summary of peer-review publications in the detection of SARS-CoV-2 RNA in air in the patient areas of hospitals

Ref.	Country [publication]	Setting [ACH, if mentioned]	Air sampler, [duration / volume of air, if mentioned]	Detection of SARS-CoV-2 RNA in air [Ct of VL in air, if mentioned]	Patient viral load (Ct or VL)	Mask for patients
1	Singapore [May 2020]	AIIR [ACH: 12]	NIOSH aerosol sampler; [4 h / 5040 L]	2 of 3 (66.7%) AIIR; [916 to 2000 copies /m^3]	Ct: 18.45 to 20.11	NM
2	China [Jun 2020]	Patient areas in two hospitals [a]	Presterilized gelatin filters with pore size 3 μm	63.6% (7/11) samples; [1 to 113 copies /m^3]	NM	NM
3	US [Jun 2020]	A clinic	Laminar-flow water vapor condensation; [1 h / 390 L]	50% (1/2) samples; [0.87 copies / L]	NM	NM
4	China [Jul 2020]	ICU [ACH: 12] & GW [ACH: 8]	SASS 2300 wetted wall cyclone sampler; [30 min / 9000 L]	35% (14/40) of ICU samples and 12.5% (2/16) of GW samples	NM	Yes
5	US [Jul 2020]	AIIR [ACH: >12]	Sartorius airport MD8 air sampler; [15 min / 750 L]	80.0% (32/40) samples; [average: 2.99 copies/L of air] [b]	NM	NM
6	UK [Jul 2020]	Patient cares in hospital	Coriolis μ air sampler; {NM]	12.5% (2/16) samples; [404 to 7048 copies /m^3]	NM	NM
7	China [Oct 2020]	ICU & isolation ward	Air sampler not specified; [1 h / 300 L]	8.3% (1/12) samples [c]	NM	Yes
8	Italy	ICU	MD8 airport portable air	100% (20/20) samples in patient	NM	NM

	[Nov 2020]		sampler; [40 min / 2000 L]	corridor & contaminated zone		
9	China [Nov 2020]	ICU & isolation ward	Two-stage cyclonic bioaerosol sampler; [4 h / 840 L]	1 sample in ICU [Ct: 41.5] & 3 samples in isolation ward [Ct: 35.6 to 44.6]	VL: 1.7 to $7x10^7$ copies/ml	Yes
10	Iran [Dec 2020]	ICU & other clinical areas	Liquid impinge biosampler; [NM]	14.3% (2/14) samples [d]	NM	NM
11	Singapore [Jan 2021]	AIIR [ACH: 12]	BioSpot-VIVAS BSS300-P bioaerosol sampler; [NM]	50% (6/12) samples; [Ct: 32.62 to 38.36] [e]	Ct: 15.22 to 20.53	NM
12	US [Feb 2021]	ICU & medical ward [ACH: >6]	Sartorius MD8 airscan sampler; [20-40 min / 1000-4000 L]	14.2% (2/14) samples; [Ct: 33.04 to 36.27]	NM	NM
13	India [Apr 2021]	ICU, medical & emergency ward	Total suspended particulate air sampler; [1 h / 90 L to 1620 L]	60% (54/90) samples; [Ct 16.11 to 32.5] [f]	NM	NM

Note. ACH, air change per hour; AIIR, airborne infection isolation room; Ct, cycle threshold value; GW, general ward; ICU, intensive care unit; NM, not mentioned; VL, viral load.

[a] Renmin Hospital and Fangcang Hospital in Wuhan, China.

[b] Analysis of data from the supplementary information.

[c] The positive air sample was collected within 10 cm of a patient who was undergoing endotracheal intubation for mechanical ventilation.

[d] Two positive air samples were collected in ICU.

[e] Virus culture from air samples was negative.

[f] Air samples collected from nursing station area separated from the patients by glass wall were excluded for analysis.

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