Letter to the Editor

Detecting Environmental Contamination of Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Isolation Wards and Fever Clinics*

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Coronavirus disease 2019 (COVID-19) is an acute respiratory disease with an incubation period of 1 to 14 d, which is caused by a novel coronavirus (SARS-CoV-2), which has spread throughout China and worldwide. Different from SARS, MERS, and influenza, the dominant clinical manifestations of COVID-19 are fever and cough, whereas upper respiratory symptoms and gastrointestinal symptoms are rare. Moreover, the elderly and those with underlying diseases become more seriously ill after infection[1]. SARS-CoV-2 is highly transmissible in humans and is characterized by a risk of nosocomial transmissions[2]. Compared with the general population, healthcare workers (HCWs) are a special population who have a particularly higher risk of acquiring COVID-19 infection because they are exposed to biological risk factors during their daily work. Specifically, COVID-19 spreads through close-range contact with patients *via* respiratory droplets and secretions, and also through direct contact but with a low infective dose[1]. Additionally, aerosol, fecal-oral, and bloodborne transmission of SARS-CoV-2 have also been considered potential threats to the safety of HCWs, even though the possibility of these transmission routes remain controversial[3]. It has been reported that many first responder HCWs worldwide have been infected with COVID-19 since the disease outbreak[4,5]. However, the transmission mode and extent of environmental contamination of SARS-CoV-2 have not been fully investigated. Therefore, this study was conducted in isolation wards and fever clinics during the Shenzhen outbreak to examine whether environmental contamination of air and surfaces could explain the ongoing risk of nosocomial transmission of SARS-CoV-2.

Using novel air sampling techniques and conventional surface swabbing, samples were obtained from Shenzhen Nanshan People's Hospital and the Third People's Hospital of Shenzhen. The Third People's Hospital of Shenzhen is the only hospital designated to treat COVID-19 patients in Shenzhen. Air sampling was performed in the waiting rooms and an outpatient room of fever clinics in Nanshan People's Hospital on March 11, 2020, using two types of bioaerosol samplers. The Coriolis micro air sampler (Bertin Technologies) is a cyclone-based air sampler and collected air for 30 min at 300 L/min into a conical vial containing 15 mL PBS buffer[6]. The Sartorius MD8 microbiological sampler collected air for 60 min at 50 L/min, with a gelatin membrane filter[7]. After the room had been sealed for 20 min, both samplers were set 1.2 m above ground for sampling.

The same samplers and methods were applied to the air sampling performed in four isolation wards in the Third People's Hospital of Shenzhen between March 13 and 14, 2020. Additionally, the samplers were set 0.5 m from the patients, who were requested to not wear masks and to cough periodically during sampling. In parallel, environmental swabs were also aseptically collected from frequently touched surfaces in wards using medical cotton swabs premoistened with Hanks solution. Samples were collected before routine cleaning. For isolation wards, cleaning is performed every 4 h with 2,000 mg/L chlorine disinfectant for high-touch areas (including floor and table), and with 75% alcohol for ventilators and other instruments.

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For fever clinics, cleaning was performed twice daily for 1 h each time, using ultraviolet light (Philips TUV 36W/G36 T8). Air conditioning filter swab samples and 200 mL condensate water samples were also collected from the air conditioner room of the floor with negative-pressure wards.

Immediately after collection, the samples were stored in biohazard logistic boxes, couriered to a biosafety level 3 (BSL-3) laboratory, and stored at -80 °C. Specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was completed within 24 h, The assay that was used to detect the presence of SARS-CoV-2, involved amplifying two different targets within the SARS-CoV-2 genome, the *ORF1ab* gene (with high specificity) and *N* gene (with high sensitivity)[8]. Briefly, after RNA extraction, 5 µL of viral RNA extracted from the sample was added to the RT-PCR mixture, which contained 12 µL of the nucleic acid amplification reaction solution (magnesium ion and nucleotide mixture), 4 µL of the enzyme mixture (DNA polymerase, reverse transcriptase, UNG enzyme), and 4 µL of the ORF1ab/N reaction solution (primers and probes for the *ORF1ab* and *N* genes and the internal reference gene RNase P), in a final volume of 25 µL. For RT-PCR against the *ORF1ab* gene, the following primers were used: forward, 5′-CCCTGTGGGTTTTACACTTAA-3′; reverse, 5′-ACGATT GTGCATCAGCTGA-3′; and probe, 5′-FAM-CCGTCTG CGGTATGTGGAAAGGTTATGG-BHQ1-3′. For RT-PCR against the *N* gene, the following primers were used: forward, 5′-GGGGAACTTCTCCTGCTAGAAT-3′; reverse, 5′-CAGACATTTTGCTCTCAAGCTG-3′; and probe, 5′-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3′. Cycle threshold (*Ct*) values, which are the minimum number of cycles required to generate a fluorescent signal that can be detected, provide quantitative information for SARS-CoV-2 that correlate with viral load in an inversely proportional relationship. The thermocycler conditions used were as follows: 50 °C for 10 min for reverse transcription; 95 °C for 5 min for initial denaturation, and then 40 cycles of 95 °C for 10 s, 55 °C for 40 s for the denaturation, amplification, and detection of fluorescence. The result was considered positive if both target were positive (*Ct* value ≤ 38), and suspiciously positive if only one target test result. Positive controls (virus-like particles specific to the RNase P genes and plasmids containing ORF1ab and N-specific fragments) and negative controls (saline) were also set up to avoid misjudging the results.

Patient data, including date of onset, symptoms, severity of illness, and the most recent SARS-CoV-2 PCR clinical test results of patients prior to sampling, were collected in all wards where environmental sampling was performed. Patient informed consent and Institutional review board approval were obtained before the environmental samplings were conducted, and the robustness of the experimental protocol was examined in the laboratory.

Four types of facility were sampled, including a waiting room for fever clinics and an outpatient room in Nanshan People's Hospital, four patient rooms and an air conditioner room on the floor with the negative-pressure wards in Shenzhen Third People's Hospital. Characteristics of the patients and their illness at the time of sampling are summarized in Table 1.

Table 1. Summary of patient symptoms at the time of sampling

Patient	Number of days from the date of sampling to the date of onset	Presence of symptoms during sampling	Symptoms	Severity of illness[a]	Number of days from the date of environmental sampling to the date of clinical detection	Most recent positive SARS-CoV-2 RT-PCR clinical samples
Patient A	10, 11	Yes	Fever, chills, and cough (with sputum)	Severe	4, 5	Nasopharyngeal swab, sputum samples
Patient B	11, 12	Yes	Fever, chills myalgia, and fatigue	Mild	2, 3	Nasopharyngeal swab, anal swab
Patient C	56, 57	No	Fever, cough (with sputum), sore throat	Mild	2, 3	Nasopharyngeal swab, sputum samples
Patient D	43, 44	Yes	Fever, cough (with sputum), myalgia, and sore throat	Mild	2, 3	Sputum samples

Note. SARS-CoV-2, acute respiratory syndrome coronavirus 2; RT-PCR, reverse transcriptase–polymerase chain reaction. [a]Disease severity was considered severe if both lungs were involved and oxygen supplementation was needed.

The results of environmental sampling tests for SARS-CoV-2 by RT-PCR are shown in Table 2. Among the 12 air samples collected from Shenzhen Third People's Hospital and Nanshan People's Hospital using either Coriolis micro air sampler (Bertin Technologies) or the Sartorius MD8 microbiological sampler, all were PCR-negative for SARS-CoV-2. For environmental surface swab samples from isolation wards, samples from 24 environmental surfaces that were frequently contacted by 4 patients were collected. Only one (4%) of the sampled sites returned positive results, which was the mask of a patient with a *Ct* values of 33 and 31 for the *ORF1ab* and *N* genes. One (4%) of the sampled sites returned suspiciously positive results, the oxygen pump tube by the patient's bed, which had a relative higher *Ct* value of 37 for the *ORF1ab* gene compared to the value of the positive mask sample, while a *Ct* value for the *N* gene was undetected. Both positive and suspiciously positive samples were taken from patient A, whose disease severity was considered severe because both lungs were involved and oxygen supplementation was needed. Furthermore, the disease severity of patients B, C, and D were relatively mild given that those patients did not require oxygen supplementation. Fifteen surface swab samples of these patients, which were collected at patient isolation wards returned negative results. Moreover, air conditioning filters and condensate water collected from the air conditioner room on the floor with negative-pressure wards were negative.

The positive swab sample from the mask demonstrated the possibility of transmitting SARS-CoV-2 *via* human respiratory droplets. Viral droplets formed by patients coughing and sneezing or asymptomatic infections can cause disease transmission if inhaled at close range. Furthermore, SARS-CoV-2 RNA was only detected on the mask of a patient when sampling was performed within a few days of symptom onset (patient A), compared with when other patient were investigated. This is consistent with the findings of an observational cohort study, which found that the viral load of SARS-CoV-2 in saliva samples was highest during the first week of symptom onset and then gradually declined[9]. The detection of SARS-CoV-2 RNA on oxygen pump tubes indicated that particular attention should be paid to the cleaning of treatment equipment because cleaning may intentionally be less thorough due to its shape and moisture sensitivity. Compared with the *Ct* value of the mask of the same patient (33 for *ORF1ab*), the *Ct* value of the oxygen pump tube was greatly increased (37 for *ORF1ab*), i.e., the viral load is lower, which highlighted the necessity of using masks for preventing public health crises and protecting medical workers. Generally, undetectable or very low rates of positivity were found for all environmental samples, including those in all high-touch areas, which showed the effectiveness of cleaning measures taken by hospitals. Air samples taken by wet air sampling and dry air filtering were both negative. This may be caused by the concentration of aerosolized virus being too low to be detected effectively, because the wards and fever clinics were well ventilated, and the patient density was low at the time of sampling. The possibility of aerosol transmission needs to be further studied. There were several limitations to this study. First, for suspected positive clinical results, repeated sampling and testing are required for confirmation. However, it is not applicable for environmental samples due to the non-repeatable characteristics of environmental sampling. Second, only a limited number of wards and fever clinics were investigated because of operational limitations. Third, viral culture was not performed on positive samples to demonstrate their viability.

This study may explain the apparent risk of nosocomial infection in countries where high level protection masks are insufficient. Adopting effective sanitization for high-risk areas and using high level protection masks for medical workers who have direct contact with high risk areas or COVID-19 patients are essential to ensure the safety of healthcare workers.

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Contributors CHEN Guo Min and JI Jia Jia designed this study and supervised all experiments; CHEN Guo Min, JI Jia Jia, JIANG Shuai, XIAO Ya Qi, and YU Shu Yuan conducted the environmental sampling; ZHANG Ren Li, HUANG Da Na, and LIU Hui completed the RT-PCR analysis; CHEN Guo Min and JI Jia Jia acquired patient data; CHEN Guo Min drafted the manuscript; JI Jia Jia and YU Shu Yuan critically revised the manuscript.

Conflict of Interest Statement The authors declare that there is no conflict of interest in the present

Table 2. Summary of environmental sampling results for SARS-CoV-2 using RT-PCR

Type of facility	Environmental sample type and SARS-CoV-2 RT-PCR results		
	Sites	No.positive/No.negative	Cycle threshold value
Shenzhen Third People's Hospital			
Patient A room	Air (MD 8)[a]	0/1	–
	Air (coriolis micro)[b]	0/1	–
	Mobile phone	0/1	–
	Tooth brush	0/1	–
	Sickbed fence	0/1	–
	Mask	1/1	33, 31
	Oxygen pump tube	1/1	37, ND
	Floor	0/1	–
	Sheets	0/1	–
	ECG Monitor	0/1	–
	Face shield[c]	0/1	–
Patient B room	Air (MD 8)	0/1	–
	Air (coriolis micro)	0/1	–
	Oxygen flowmeter	0/1	–
	Kettle handle	0/1	–
	Mobile phone	0/1	–
	Water cup	0/1	–
	Mask	0/1	–
Patient C room	Air (MD 8)	0/1	–
	Air (coriolis micro)	0/1	–
	Mask	0/1	–
	Floor drain	0/1	–
	Toilet squatting	0/1	–
	Floor	0/1	–
	Mobile phone	0/1	–
Patient D room	Air (MD 8)	0/1	–
	Air (Coriolis micro)	0/1	–
	Mask	0/1	–
	Floor drain	0/1	–
	Toilet squatting	0/1	–
	Floor	0/1	–
	Mobile phone	0/1	–
Air conditioning unit plant room	Air conditioning filter	0/1	–
	Condensate water	0/1	–
Shenzhen Nanshan People's Hospital			
Waiting hall for fever clinics	Air (MD 8)	0/1	–
	Air (coriolis micro)	0/1	
Outpatient room	Air (MD 8)	0/1	–
	Air (coriolis micro)	0/1	

Note. SARS-CoV-2, acute respiratory syndrome coronavirus 2; ECG, electrocardiogram; ND, not detected; RT-PCR, reverse transcriptase-polymerase chain reaction. [a]The volume of each air sample taken by the Sartorius MD8 microbiological sampler consisted of 300 L of air. [b]The volume of each air sample taken by the coriolis micro air sampler consisted of 9,000 L of air. [c]The swab of a face shield was taken from a medical worker who had been working in the room of patient A for 4 h.

study.

Patient Informed Consent Obtained.

Ethics Approval Ethical and institutional review boards for human investigation at Shenzhen Center for Disease Control and Prevention.

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