Air and surface contamination by SARS-CoV-2 virus in a tertiary hospital in Wuhan, China

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ABSTRACT

Background: Few studies have explored air and surface contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in healthcare settings.
Methods: Air and surface samples were collected from the isolation wards and intensive care units designated for coronavirus disease 2019 (COVID-19) patients. Clinical data and the results of nasopharyngeal specimen and serum antibody testing were also collected for the patient sample.
Results: A total of 367 air and surface swab samples were collected from the patient care areas of 15 patients with mild COVID-19 and nine patients with severe/critical COVID-19. Only one air sample taken during the intubation procedure tested positive. High-touch surfaces were slightly more likely to be contaminated with SARS-CoV-2 RNA than low-touch surfaces. Contamination rates were slightly higher near severe/critical patients than near mild patients, although this difference was not statistically significant ($p > 0.05$). Surface contamination was still found near the patients with both positive IgG and IgM.
Conclusions: Air and surface contamination with viral RNA was relatively low in these healthcare settings after the enhancement of infection prevention and control. Environmental contamination could still be found near seroconverted patients, suggesting the need to maintain constant vigilance in healthcare settings to reduce healthcare-associated infection during the COVID-19 pandemic.

Introduction

Following its first emergence in December 2019 in Wuhan City, China, the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread to over 200 countries and regions within 5 months (Phelan et al., 2020). As of June 30, 2020, the total number of cases of coronavirus disease 2019 (COVID-19) has reached over 10 million globally, and the death toll has reached nearly 500,000 (Anon, 2020). Similar to two previous coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) (Ip et al., 2004) and Middle East respiratory syndrome coronavirus (MERS-CoV) (Hunter et al., 2016), this newly emerged virus has caused outbreaks in healthcare settings (Chan et al., 2020). The rapid spread of SARS-CoV-2 infection could have been facilitated by transmission from mild, pre-symptomatic, or even asymptomatic cases (Kam et al., 2020; Li et al., 2020; Rothe et al., 2020), suggesting that the early detection of cases might be a challenge in healthcare settings. Studies have shown that viral shedding may peak soon after symptom onset (Wölfel et al., 2020), and the viral loads of asymptomatic COVID-19 patients could be as high as those of symptomatic patients (Zou et al., 2020).

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Similar to SARS-CoV, aerosols of the novel coronavirus SARS-CoV-2 have been found to survive in air for up to 3 h and on plastic and stainless steel surfaces for up to 72 h in a controlled experimental environment (van Doremalen et al., 2020). The RNA of SARS-CoV-2 has been detected in respiratory specimens, feces, blood, and urine samples (Wang et al., 2020; Young et al., 2020). Previous studies have reported that viral shedding of SARS-CoV-2 peaks soon after symptom onset, and peaks within 1 week (Wolfel et al., 2020). Most patients have seroconverted within 2 weeks, and seropositivity of IgG appears slightly earlier than that of IgM (To et al., 2020). Current evidence also suggests that serum antibody levels might not be associated with disease severity, but it remains unclear whether viral shedding could be lower among those with both elevated IgM and IgG (To et al., 2020).

In this study, air and surface samples were collected from isolation wards and the intensive care unit (ICU) of a tertiary hospital in Wuhan, with the aim of evaluating environmental contamination after the enhancement of infection prevention and control measures (IPC) during the COVID-19 pandemic. Furthermore, the associations of patient disease severity, seroconversion status, and environmental contamination were assessed.

Methods

Study site

Surveillance data were obtained from a single tertiary hospital in Wuhan, the Optics Valley Branch (OVB) of Tongji Hospital, Huazhong University of Science and Technology. This hospital had 828 beds designated for patients with severe and critical COVID-19 pneumonia. The diagnostic criteria for COVID-19 pneumonia followed the guidelines of the National Health Commissions of China (New Coronavirus Pneumonia Prevention and Control Program, 2020). During the period February 2 to March 30, 2020, a total of 1462 patients were admitted to this hospital and 1341 were cured. Eight hundred healthcare personnel (HCP) are employed by this hospital; they were joined in early February by 2393 HCP deployed from 17 other hospitals outside of Wuhan to resolve the manpower shortage. By April 23, 2020, when all of the COVID-19 patients had been discharged or transferred from this hospital, none of these HCP was infected with SARS-CoV-2.

Infection prevention and control measures

Areas across the entire hospital were classified into low- and high-risk areas, with different IPC measures implemented. The latter included triage stations, fever clinics, outpatient clinics and wards for respiratory and infectious diseases, and the emergency department. The rest were classified as low-risk areas. HCP including doctors, nurses, and ward assistants were required to put on a full set of personal protective equipment (PPE) when working in high-risk areas, whereas only surgical masks were required for those working in low-risk areas. Details of the requirements for PPE can be found in Supplementary Material Table S1. The layout of an isolation ward in this hospital is shown in Supplementary Material Figure S1. It was recommended that HCP enter anterooms in pairs so that they could monitor and assist each other when removing PPE. Clear instructions for PPE removal sequences, as well as reminders regarding disinfecting door handles when opening doors, were posted in these anterooms. Patients were provided with a medical mask and requested to keep wearing it if tolerable. They were advised to change their mask every 4 h.

There were no airborne infection isolation rooms (AIIR) in this hospital. To reduce the risk of airborne transmission, the central air conditioning system was turned off and natural ventilation was used in isolated wards. Windows were kept open for 30 min at least twice per day, and one electronic fan was installed on the top of the windows in each inpatient ward to increase ventilation. If there were no patients inside the room, ultraviolet lights (wavelength 253.7 nm, SX-01A; Shuangsheng Medical Ltd) were used to disinfect empty isolation rooms for at least 1 h. In clean areas (green zone in Supplementary Material Figure S1), this was followed by the application of 3% hydrogen peroxide spray and the room was then closed for 2 h for disinfection.

Surfaces of the premises and the floors were disinfected twice per day using sodium hypochlorite at 1000 mg/l. In the event of a spillage, sodium hypochlorite at 5000 mg/l was used to disinfect soiled surfaces or the floor. In the event of a large spillage of blood, vomit, or other body fluids, the soiled surfaces or floor were immediately covered with sodium hypochlorite at 5000 mg/l for 30 min, followed by disinfection with sodium hypochlorite at 1000mg/l.

Collection of air and surface samples

During the period March 14–29, 2020, the IPC team of OVB hospital conducted a comprehensive investigation on environmental contamination with SARS-CoV-2. The selection of patients for the present study was subject to patient consent and the availability of manpower. To investigate the contamination risks of aerosol-generating procedures, we particularly selected at least one patient who was receiving one of the following means of oxygen therapy at the time of sampling: oxygen supply via nasal cannula, invasive ventilation via tracheostomy, invasive ventilation via endotracheal intubation, and extracorporeal membrane oxygenation (ECMO). Additional patients with mild and severe disease were then recruited from different patient rooms depending on the availability of manpower and testing kits. A total of 24 patients, 15 in the general isolation wards and nine in the ICU, were selected from 11 wards. Anonymized demographic and clinical data of these patients were collected from the electronic medical records of OVB hospital.

In the general isolation wards, three patients stayed in one room and were advised not to walk around except when going to the bathroom. The most severe/critical patients stayed in single rooms in the ICU. The individual patient data and a floor plan of the sampling sites in the ICU can be found in the Supplementary Material file. Surface samples were obtained before the daily decontamination procedures were implemented. Experienced infection control nurses who wore full PPE swabbed selected high-touch surfaces, including patient mobile phones, bedrails, door handles, light switches, side tables, and medical instruments in the patient wards, as well as low-touch surfaces including floors and chairs in the corridor. Surface sampling was conducted before the routine cleaning procedures. Each surface was sampled with two pre-moistened sterile cotton swabs simultaneously. Both were immediately placed into a single tube of viral transport medium (VTM; Yocon Ltd, Beijing, China). Air samples were obtained by placing an air sampler within 1 m of the patient's head; this continuously filtered air at a speed of 5 l/min and trapped small virus particles on a membrane. After 1 h the membrane was removed and cut into small pieces to be stored in VTM prior to further testing. The air sampler was placed at the same height as (or slightly lower than) an electronic fan installed on top of the windows to expel the air from the wards to the outside. Air samples were obtained from patient rooms, the corridor outside the patient rooms, and in the nearby nursing stations.

Sample collection from patients and HCP

Hand swabs were collected from both hands of patients with mild disease. The hands of patients with severe and critical disease

were not swabbed due to their condition. The outer and inner layers of surgical masks worn by these patients were cut into small pieces that were immediately placed in VTM for later laboratory testing. Body fluid samples including sputum and alveolar lavage fluid were also obtained from some of the severe and critical patients, and saliva was taken from an additional 31 mild/moderate patients who were not sampled for environmental contamination.

The nurses and doctors who were taking care of these patients were also invited to participate in this study. Infection control nurses swabbed the surfaces of their PPE, including coveralls (front and arm side), face-piece (front surface), gloves, and bottoms of shoe covers. Hand swabs were also collected from some HCP before they performed hand hygiene. An average of five samples were obtained from each HCP. Nurses also recorded the time since removing PPE, exposure to aerosols, and incidence of spillover, if any.

Laboratory tests and data analysis

Samples stored in VTM were immediately transported on ice to the laboratory of the BGI Medical Diagnostics Company (Wuhan) for RT-PCR testing of the open reading frame (ORF) 1a/b genes of SARS-CoV-2. RNA was extracted using the QIAamp Viral RNA Mini Kit, and then an RT-PCR kit was used (BGI Biotechnology, Wuhan) in a SLAN Realtime PCR system (Hongshi Technology, Shanghai, China). The test results were categorized by high- and low-touch surfaces near mild and severe/critical patients. Blood samples were obtained from the patients on the same day for testing for SARS-CoV-2-specific IgM and IgG antibodies using kits from Wondfo Biotech Co., Ltd (Guangzhou, China); these had a sensitivity of 86.4% and specificity of 99.6% for IgG and IgM (Wondfo, 2020). A titer higher than 10 AU/mL was regarded as positive. The classification of mild or severe/critical infection followed the national diagnosis criteria (New Coronavirus Pneumonia Prevention and Control Program, 2020). Surface contamination rates were compared between the mild and severe/critical patient groups, and between seroconversion groups (IgM or IgG positive) using Fisher's exact test. The level of significance was set to 0.05.

Ethical considerations

Ethical approval was obtained from the Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology.

Results

A total of 355 surface swab samples were collected from low-/high-touch surfaces near patients, hands and masks of patients, and PPE of HCP while taking care of these patients. Details of the sampling sites are given in Supplementary Material Table S2. One low-touch surface sample was found to be positive for SARS-CoV-2 and nine high-touch surfaces were found to be positive. High-touch surfaces near severe/critical patients had a slightly higher contamination rate than those near mild patients (5.7% vs 2.4%, Table 1). Eight positive environmental samples were from four severe/critical patients at 22–43 days post-onset, of whom two were IgG-positive; the rest were both IgM- and IgG-positive. Two positive high-touch surface samples were collected from two mild patients, and both seroconverted (IgM- and IgG-positive), one at 35 days and the other at 48 days post symptom onset. One of them also had SARS-CoV-2 contamination on their hands, but tested negative in throat swabs on the same day.

Of the 24 patients, 18 were positive for both IgM and IgG, five were positive for IgG only, and one was negative for both. Ten of 23 (43.5%) patients who seroconverted still had positive RT-PCR test results on throat specimens. The surface contamination of the IgM/IgG-positive group was slightly lower than that of the IgG-positive only group (3.1% vs 5.3%, *p* = 0.358). Similar contamination rates were observed between the PCR-positive and negative groups (3.1% vs 4.0%, *p* = 0.775) (Table 1).

Of 40 environmental samples from fever clinics and ICU common areas (corridors and nursing stations), none tested positive. All 20 swabs of door handles and keyboards and 17 air samples in clean areas also tested negative. Twelve air samples were collected from patient rooms, one from near the air exhaust fan on the window and the rest within 1 m of patient heads. Only one sample was positive for SARS-CoV-2, which was collected within 10 cm of a female patient who was undergoing

Table 1
Comparison of SARS-CoV-2 contamination among the environmental samples taken from patients with mild/moderate and severe/critical COVID-19.

Sample	Grouped by disease severity		Grouped by seroconversion			Grouped by PCR tests	
	Mild (*n* = 15)	Severe/critical (*n* = 9)	IgM(+)/IgG(+) (*n* = 18)	IgM(−)/IgG(+) (*n* = 5)	IgM(−)/IgG(−) (*n* = 1)	PCR(−) (*n* = 14)	PCR(+) (*n* = 10)
Days from onset (median, IQR)	45 (36–50)	48 (36–52)	48 (43–52)	35 (34–47)	22 (22–22)	47.0 (35–52)	46 (37–48)
Positive RT-PCR test for throat specimen, positive/total (%)[a]	7/15 (46.6%)	3/9 (33.3%)	8/18 (44.4%)	2/5 (40.0%)	0/0 (0.0%)	–	–
Body fluids, positive/total (%)							
Sputum	–	2/5 (40.0%)	1/1 (100.0%)	1/4 (25.0%)	–	0/3 (0.0%)	2/2 (100.0%)
Saliva[b]	1/31 (3.2%)	1/5 (20.0%)	1/35 (2.9%)	0/1 (0.0%)	–	1/1 (100.0%)	1/35 (2.9%)
Air near patients, positive/total (%)	0/2 (0.0%)	1/10 (10.0%)	0/4 (0.0%)	1/8 (12.5%)	–	1/6 (16.7%)	0/6 (0.0%)
Low-touch surfaces, positive/total (%)	0/17 (0.0%)	1/22 (4.5%)	1/25 (4.0%)	0/13 (0.0%)	0/1 (0.0%)	1/22 (4.5%)	0/17 (0.0%)
High-touch surfaces, positive/total (%)	2/83 (2.4%)	7/122 (5.7%)	5/140 (3.6%)	4/61 (6.6%)	0/4 (0.0%)	6/119 (5.0%)	3/86 (3.5%)
Hands of patients, positive/total (%)	2/14 (14.3%)	–	2/12 (16.7%)	0/2 (0.0%)	–	2/7 (28.6%)	0/7 (0.0%)
Masks, positive/total (%)	0/28 (0.0%)	0/8 (0.0%)	0/32 (0.0%)	0/4 (0.0%)	–	0/18 (0.0%)	0/18 (0.0%)
Ventilator circuit, positive/total (%)	–	1/7 (14.3%)	0/4 (0.0%)	1/3 (33.3%)	–	0/6 (0.0%)	1/1 (100.0%)
PPE of HCP, positive/total (%)	–	0/54 (0.0%)	0/42 (0.0%)	0/12 (0.0%)	–	0/52 (0.0%)	0/2 (0.0%)
Total surface sample, positive/total (%)[c]	4/142 (2.8%)	9/213 (4.2%)	8/255 (3.1%)	5/95 (5.3%)	0/5 (0.0%)	9/224 (4.0%)	4/131 (3.1%)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; IQR, interquartile range; PPE, personal protective equipment; HCP, healthcare personnel. Fisher's exact test: *p*-value = 0.577 between mild patients and severe/critical patients; *p*-value = 0.358 between patients with both antibodies positive (IgM(+)/IgG(+)) and only IgG-positive (IgM(−)/IgG(+)); *p*-value = 0.775 between patients with PCR test positive and PCR test negative.
[a] The test result on the day of environmental sampling or 2 days before and after the sampling date.
[b] Thirty-one patients only had saliva samples tested.
[c] Total surface included low-touch surfaces, high-touch surfaces, hands of patients, masks, ventilator circuit, and PPE of HCP.

endotracheal intubation for invasive mechanical ventilation. One sample of cooling water from ventilator circuits was positive, suggesting the need for regular thorough cleaning of the ventilator. Two of nine severe or critical patients had sputum and saliva that tested positive, and one saliva sample from 31 mild/moderate patients tested positive. All three also had SARS-CoV-2 detected in their throat samples on the same day.

SARS-CoV-2 RNA was not detected on any of the 36 surgical masks from 18 patients (14 mild and four severe/critical), although some of the patients had worn the same mask for 24 h. Regarding the swabs from gloves, gowns, face-pieces, and the bottoms of boot covers worn by HCP inside unclean areas, all 54 samples tested negative. None of the hand swabs from HCP tested positive.

Discussion

In this study, a large number of surface swabs were collected from various sites in isolation wards and the ICU after enhanced standard and transmission-based precautions. Environmental contamination of low- and high-touch surfaces, patient hands, and PPE of HCP were also compared, and the results were linked to the clinical data of the sample of patients. A small proportion of samples (2.8%) were positive for SARS-CoV-2 by RT-PCR, which is much lower than the proportion reported in an emergency field hospital in Wuhan, China (Guo et al., 2020). The reasons for this difference could be the stringent IPC measures adopted in the OVB hospital. Nevertheless, a slightly higher contamination rate was observed on high-touch surfaces than on low-touch surfaces, suggesting that environmental decontamination should focus more on these high-touch surfaces.

It was observed that severe/critical patients were slightly more likely to contaminate their surroundings as compared to mild patients. Most of these patients were 20 days post symptom onset, and 10 of the 24 patients (41.7%) still tested positive for SARS-CoV-2 by throat swab on the day of sampling. The serology tests prior to or on the sampling date showed that nearly all had seroconverted (23/24 IgG > 10 Au/mL, 18/24 IgM > 10 Au/mL). This echoes the findings of a recent study performed in Germany (Wölfel et al., 2020), which found that viral shedding continued after seroconversion. A recent study reported that SARS-CoV-2 RNA could be detected in feces for as long as 47 days (Wu et al., 2020). Unfortunately, we could not determine whether viruses detected on surfaces were still viable, due to the lack of laboratory capacity for viral culture and quantitative PCR. Therefore, it is unclear whether environmental contamination was correlated with patient viral load.

SARS-CoV-2 RNA could be detected in the saliva and sputum of three patients (one severe/critical patient had both saliva and sputum positive), which is consistent with previous reports (Pan et al., 2020). Surprisingly, none of the surgical masks worn by patients had positive results. Another study found only one out of 14 surgical masks worn by mild and severe COVID-19 patients tested positive for SARS-CoV-2 (Guo et al., 2020). This low positive rate is not significantly different from that of the present study. It is speculated that the reason for the negative results could be due to low virus titers in these patients, as most of them were 30–40 days post symptom onset when sampled. Laboratory studies have shown that SARS-CoV-2 virus titers peak at 5–6 days post symptom onset and decrease to an undetectable level at 8–10 days post symptom onset in most patients (He et al., 2020; Pan et al., 2020; Wölfel et al., 2020). Several studies have reported a longer period of detecting RNA by RT-PCR in biological samples (particularly feces), compared to detecting viable viruses by viral culture (Wölfel et al., 2020; Wu et al., 2020). In this study, only one patient had diarrhea, but none of the samples from the patient were positive, including five samples from bathroom surfaces.

A study by Liu et al. collected air samples from different areas in one tertiary hospital and one Fangcang shelter hospital in Wuhan; Fangcang shelter hospitals served as quarantine centers for mild COVID-19 cases with limited medication treatment (Chen et al., 2020). SARS-CoV-2 RNA was detected at low concentrations in the Fangcang hospital, but not in the patient rooms of the tertiary hospital (Liu et al., 2020). Another study in the AIIR of a tertiary hospital in Singapore also did not detect any virus in air samples (Ong et al., 2020a). Similarly, in the present study, only one air sample that was collected near a patient during the endotracheal intubation procedure had SARS-CoV-2 RNA detected. No virus was detected in an additional 17 air samples from clean areas (staff offices), although isolation wards were not under negative pressure. The study findings indicate that natural ventilation together with extra air exhaust fans could efficiently reduce virus aerosols in patient rooms. Of note, five surface swabs from the front side of face-pieces and gloves of HCP who conducted aerosol-generating procedures were all negative. This highlights the importance of wearing proper PPE while performing aerosol-generating procedures.

Previous SARS, MERS, and pandemic influenza H1N1 outbreaks have demonstrated the importance of contact and droplet precautions in the prevention of healthcare-associated infections during the outbreak of emerging respiratory viruses (Al-Tawfiq and Memish, 2015; Cheng et al., 2010; Holden and Mogck, 2003). Based on the lessons from the SARS and MERS outbreaks and the potential airborne transmission routes of SARS-CoV-2, the national infection prevention and control guidelines of the National Health Commission of China have recommended the implementation of contact, droplet, and airborne precautions in addition to standard precautions (Infection Prevention and Control Guidelines for Novel Coronavirus in Healthcare Settings, 2020). The PPE requirements are summarized in Supplementary Material Table S1.

Although a large number of surface swabs were collected from different parts of PPE, including the bottoms of boot covers, none tested positive by PT-PCR. Another study found a high contamination rate in three swabs from the soles of shoes of HCP, but none of the samples from other parts of the PPE were positive (Guo et al., 2020). Interestingly, studies in Singapore detected SARS-CoV-2 in surface swabs of the fronts of shoes worn by HCP, but not in other parts of PPE (Ong et al., 2020a, b). The transmission risk from HCP to patients appears low, since none of the PPE samples (except shoes) in these studies was found to be positive. Nevertheless, more frequent floor disinfection might still be necessary to further reduce the transmission risk in healthcare settings.

The hands of two patients were found to be contaminated with SARS-CoV-2, which highlights the importance of hand hygiene education for patients. One bottle of alcohol-based hand rub was placed near each ward entrance, and the patients were taught how to properly wash or rub their hands when they were admitted. If resources allow, each patient should ideally have one bottle near their bedside. None of the HCP were found to have contaminated hands, which could have been due to regular audits on hand hygiene compliance by the IPC team.

There are several limitations to this study. First, this was a single center observational study; therefore, the results and protocol might not be generalizable to other healthcare facilities, especially those with limited resources. Second, although a large number of samples were collected, the number of patients recruited was relatively small. As a result, the statistical power might not have been sufficient for comparison across patient groups. Third, the sensitivity and specificity of RT-PCR tests on surface contamination samples might not be same as those for human specimens. Hence, false-negative and positive results might have occurred in the samples. Finally, it is unclear whether the virus on the surfaces was still viable, since the positive specimens were not cultured.

In conclusion, environmental contamination with SARS-CoV-2 RNA could be found even in seroconverted patients in healthcare settings, and the contamination risk was higher in high-touch areas near severe/critical patients. Enhanced standard and transmission-based precautions should be maintained during the entire COVID-19 pandemic period, to minimize the infection risk of HCP.

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Role of sponsors

Data sharing

All data and materials used in this work are available in the Supplementary Material file.

Conflict of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.07.027.

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