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SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus Disease 2019 outbreak in a Chinese hospital

Jie Wang[c], Haiting Feng[b], Sheng Zhang[b], Zuowei Ni[b], Lingmei Ni[b], Yu Chen[a], Lixin Zhuo[d], Zifeng Zhong[b], Tingting Qu[a,*]

[a] State Key Laboratory for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[b] Infection Control Department, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[c] Respiratory Department, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[d] General Affairs Department, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

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ABSTRACT

Objectives: The aim of this paper was to monitor the presence of SARS-Cov-2 among hospital environment surfaces, sewage, and personal protective equipment (PPE) of staffs in isolation wards in the First Affiliated Hospital of Zhejiang University, China.
Methods: Surfaces of objects were routinely wiped with 1000 mg/L chlorine containing disinfectant. Air and sewage disinfection was proceeded routinely and strictly. Hospital environmental surfaces and PPE of staffs in isolation wards were sampled using swabs. The sewage from various inlet and outlets were sampled. The respiratory and stool specimens of patients were collected. The respiratory specimens of staffs in the isolation wards were also sampled once a week. Quantitative real-time reverse transcription PCR (qRT-PCR) methods were used to confirm the existence of SARS-Cov-2 RNA. Viral culture was done for the samples positive for SARS-Cov-2 RNA.
Results: During the study period, 33 laboratory-confirmed patients were hospitalized in isolation wards in the hospital. None of SARS-Cov-2 RNA was detected among the 36 objects surface samples and 9 staffs PPE samples in isolation wards. Though the 3 sewage samples from the inlet of preprocessing disinfection pool were positive for SARS-CoV-2 RNA and the sample from the outlet of preprocessing disinfection pool was weakly positive, the sewage sample from the outlet of the last disinfection pool was negative. All of the 5 sewage samples from various points were negative by viral culture of SARS-Cov-2. None of the respiratory specimens of staffs in the isolation wards were positive.
Conclusions: Though SARS-Cov-2 RNA of the sewage samples were positive from inlets of the sewage disinfection pool and negative from the outlet of the last sewage disinfection pool, no viable virus was detected by culture. The monitoring data in this study suggested that the strict disinfection and hand hygiene could decrease the hospital-associated COVID-19 infection risk of the staffs in isolation wards.

Introduction

Since December 2019, coronavirus disease 2019 (COVID-19) emerged in Wuhan city, then rapidly spread throughout China, and was also reported in other countries (Benvenuto et al., 2020; Huang et al., 2020; Park et al., 2020; Tian et al., 2020; Xu et al., 2020; Zhang et al., 2020). The virus was subsequently renamed SARS-CoV-2 as it is similar to the coronavirus responsible for severe acute respiratory syndrome (SARS-CoV), a member of the subgenus Sarbecovirus (Beta-CoV lineage B). The transmission of SARS-CoV-2 is associated with close contact to COVID-19 patients and droplet secretions of those patients. According to a report from the China Centers for Disease Control and Prevention, up to February 24th 2020, 3387 medical staffs were confirmed with COVID-19, of which were mainly occurred in January 2019 from Hubei Province (Novel Coronavirus Pneumonia Emergency Response Epidemiology, 2020; Wang et al., 2020). The occurrence of healthcare-associated infections might be closely related to pathogens contamination

* Corresponding author at: 79# Qingchun East Road, Hangzhou 310001, China.
E-mail address: qutingting@zju.edu.cn (T. Qu).

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in the hospital environment (Beggs et al., 2015, Chowell et al., 2015). The outbreak of this novel virus placed challenges on hospital environmental hygiene. Because of the COVID-19 pandemic, the isolation wards in general hospitals have all been transformed into temporary infectious disease wards.

In the past, several studies on environmental sampling have performed to identify the contamination in field-settings with SARS or MERS-CoV (Bin et al., 2016; Otter et al., 2016). Though the data was limited, it appeared that the survival capacity of various human coronavirus was different (Chan et al., 2011; van Doremalen et al., 2013). Recently, SARS-CoV-2 RNA has been detected from a door handle in a confirmed COVID-19 patient's home by centers for disease control and prevention (CDC) in Guangzhou, China. It was also reported that SARS-CoV-2 RNA was detected in hospital environment in Singapore (Ong et al., 2020). Hence, comprehensive monitoring of hospital environmental hygiene during the outbreak of the pandemic is significant to ensure the safety of medical treatment and the quality of hospital infection control.

In order to monitor the hospital environmental hygiene and evaluate the quality of hospital infection control, we reveal the SARS-CoV-2 RNA data of environmental hygiene in the isolation wards of the First Affiliated Hospital of Zhejiang University.

Methods

Participant characteristics

The severe patients with COVID-19 in Zhejiang Province were collected and hospitalized in the Zhijiang Campus, First Affiliated Hospital of Zhejiang University, China. The Zhijiang Campus of the First Affiliated Hospital of Zhejiang University was transformed into temporary infectious disease hospital. Confirmed patients of COVID-19 were hospitalized in the 3 isolation wards including one Isolation Intensive Care Unit (ICU) ward and two general Isolation wards. Each isolation ward was separated into three parts and two passages. Three zones including contaminated area, semi-contaminated area, and clean area. A contaminated area is a specifically designated area for patients of COVID-19 and contaminated items such as patients' wastes. A clean area is a specifically designated area for non-contaminated items. Patients should not enter the clean area. Semi-contaminated area was set up between the contaminated area and the clean area. The items were potentially polluted can be placed in this area. Doctor's offices were placed in this area. While two passages are a passage for medical staff and a passage for patients. Buffer room is set up between a contaminated zone and a semi-contaminated zone. In each isolation ward, two buffer zones are set up for removing the staff PPEs in accordance with the transmission features of COVID-19. The suspected patients of COVID-19 were nursed in single rooms, while the confirmed patients of COVID-19 were cohorted in cubicles with bed spacing of not less than 1.2 meters. The isolation rooms were not under negative pressure because the isolation wards were reconducted temporarily.

Disinfection

Disinfection of indoor air in isolation wards of medical institutions refer to Hospital Air Purification Management Code (WS/T 368-2012) by air disinfector based on plasma. Visible contaminants on surfaces of treatment facilities and equipment including bed rails, nightstand, furniture, door handles and other household items, should be completely removed before disinfection. Surfaces of objects were routinely wiped with 1000 mg/L chlorine containing disinfectant every 4 h in Isolation ICU ward and every 8 h in general Isolation wards. Preprocessing disinfection equipment were added before sewage drainage from the isolation wards into the final sewage disinfection pool. Sodium hypochlorite was used for sewage disinfection.

Patients' samples collection

Respiratory and stool specimens collected from all patients at admission were tested by real time polymerase chain reaction for SARS-CoV-2 RNA.

Staffs' respiratory samples collection

The respiratory specimens such as naso- vs. oropharyngeal swabs or sputum samples of staffs in the isolation wards were collected once a week and tested by real time polymerase chain reaction for SARS-CoV-2 RNA.

Hospital environmental sampling and staff personal protective equipment (PPE) sites sampling

During February 19th–24th, 2020, we collected samples of the environmental surfaces in Isolation ICU ward and Isolation wards, including the clean area, the semi-contaminated area, and the contaminated area. Environmental surfaces were sampled by using ClassiqSwabs (Copan Flock Technologies, Brescia, Italy), and collected in universal transport medium (UTM) containing Hanks' Balanced Salt Solution, BSA, HEPES, amino acids, glycerin and so on. Sewage from the isolation wards were collected from various inlet and outlet were sampling. Front surface of N95 masks and gloves of staffs in isolation wards were also sampled using swabs and collected in UTM. (Table 1).

Real-time reverse transcription PCR (RT-PCR)

Laboratory confirmation of the virus was performed using real time reverse transcription polymerase chain reaction using the SARS-Cov-2 nucleic acid detection kit (Shanghai Berger Medical Technology Co., China) (Huang et al., 2020; Xu et al., 2020). Cycle threshold values, i.e., number of cycles required for the fluorescent signal to cross the threshold in RT-PCR, quantified viral load, with lower values indicating higher viral load. A sample was considered positive when the qRT-PCR Ct value was $\leq$40.

Virus isolation and culture

Virus culture must be performed in a laboratory with qualified Biosafety Level 3 (BSL-3). Samples were obtained and inoculated on Vero-E6 cells for virus culture. The cytopathic effect (CPE) was observed after 96 h. Detection of viral nucleic acid in the culture medium indicated a successful culture.

Results

Isolation wards and patients characteristics

During the period from February 19th to 24th, 2020, a total of 33 laboratory-confirmed COVID-19 patients were hospitalized Isolation ward in Zhijiang Campus, the First Affiliated Hospital of Zhejiang University, China. Among the 33 laboratory-confirmed COVID-19 hospitalized patients, 9 intensive care patients were hospitalized in Isolation ICU ward including 7 patients with mechanical ventilation and 2 patients without mechanical ventilation. The other 24 patients were hospitalized in the other two general Isolation wards. All the patients without mechanical ventilation were wearing surgical masks all the time in isolation wards.

Table 1
SARS-COV-2 RNA results of samples from the environmental, sewage, and staff PPE sites of the hospital isolation areas.

Classification		Samples	Positive samples	Cycle threshold value
Environmental sites				
Contaminated area	Isolation ICU ward	Nightstands of patient A and B	0/2	
		Monitor screens of patient A and B	0/2	
		Bed rails of patient A and B	0/4	
		Intercoms	0/2	
		Sinks	0/2	
		Personal digital assistants (PDA)	0/2	
		Door handles	0/2	
	General Isolation wards	Door handles (inside and outside)	0/4	
Semi-contaminated area	Isolation ICU ward	Door handles (inside and outside)	0/4	
		Keyboards	0/2	
	General Isolation wards	Door handles (inside and outside)	0/4	
		Keyboards	0/2	
Clean area	Isolation ICU ward	Door handles (inside and outside)	0/2	
	Isolation wards	Door handles (inside and outside)	0/2	
Sewage pools	Inlets of preprocessing disinfection pool	Sewage	3/3	29.37,30.58,32.42
	Outlet of preprocessing disinfection pool	Sewage	1/1	33.55
	Final outlet of sewage disinfection pool	Sewage	0/1	
Staff PPE sites				
	Isolation wards	Front surface of N95 masks	0/5	
		Gloves	0/4	

Note: COVID-19 confirmed patient A with mechanical ventilation; COVID-19 confirmed patient B without mechanical ventilation.

Detection of SARS-CoV-2 RNA among health-care settings, sewage, and staffs' PPE

In routine cleaning and disinfection, the 36 samples of environmental surface in isolation wards including the clean area, the semi-contaminated area, and the contaminated area were all negative. Front surface of N95 masks and gloves of staffs in isolation wards were also negative for SARS-CoV-2 RNA (Table 1).

Three sewage samples from the inlets of preprocessing disinfection equipment were positive for SARS-CoV-2 RNA (Cycle threshold value 29.37, 30.58, and 32.42). After preprocessing disinfection, the sewage sample from outlet of preprocessing disinfection pool was weakly positive (Cycle threshold value 33.55). The sewage sample from final outlet of the last sewage disinfection pool was negative. (Table 1) However, all of the 5 sewage samples were negative by viral culture.

Viral loads in respiratory and stool specimens of COVID-19 confirmed patient A and B

Patient A and B were hospitalized in Isolation ICU ward. The sampling of nightstands and bed rails were surrounding the patient A and B, respectively. During the study period, the respiratory and stool samples of patient A with mechanical ventilation were positive for SARS-CoV-2 RNA with cycle threshold value 19.85 and 21.28 respectively. While, the respiratory sample of patient B without mechanical ventilation was positive for SARS-CoV-2 RNA with cycle threshold value 28.37, but the stool sample negative for patient B.

Detection of SARS-CoV-2 RNA among staffs in the isolation wards

All of the staffs' respiratory specimens in the isolation wards were negative.

Discussion

The transmission of SARS-CoV-2 was reported to be associated with close contact to COVID-19 patients and droplet secretions of those patients (Zou et al., 2020). Stool specimens of COVID-19 patients were positive for SARS-CoV-2, suggesting that viral shedding in stool might be a potential route of transmission which was also reported in SARS previously (Wang et al., 2005). In recent studies, environmental sampling also has identified contamination in field-settings with SARS-CoV-2 (Ong et al., 2020). If SARS-CoV-2 transfers to hands or other equipment at a concentration above the infectious dose, it will initiate infection through contact with the eyes, nose or mouth by indirect contact. Thus, an effective disinfection is very necessary for hospital infection control and medical staffs' protection. With the outbreak of COVID-19 in China, the isolation wards in general hospitals have all been transformed into temporary wards in order to meet the standard of isolation wards in infectious disease hospitals. Such temporary isolation wards might have many concerns. Further environmental monitoring assessments are necessary.

During sampling process in our study, a total of 33 laboratory confirmed patients with COVID-19 were hospitalized in the isolate wards including one Isolation ICU ward and two general Isolation wards. With routine cleaning and disinfection, none of SARS-CoV-2 RNA was detected among object surfaces in isolation wards including the clean area, the semi-contaminated area, and the contaminated area. Siegel et al. reported that medical ventilators might generate respiratory aerosols that have been associated with an increased risk of occupationally acquired infection among healthcare personnel (Siegel et al., 2007). We also detected the object surfaces such as bed rails and nightstands surrounding the COVID-19 patients with or without mechanical ventilation. The two patients' respiratory specimens were both positive for SARS-CoV-2 RNA, while the stool specimen of patient A with mechanical ventilation was positive for SARS-CoV-2 RNA but that of patient B without mechanical ventilation was negative. The object surfaces closely surrounding the two patients with or without mechanical ventilation were all negative for SARS-CoV-2 RNA. The sampling time was about 4 h after objects disinfection with 1000 mg/L chlorine containing disinfectant. It indicated that the current disinfection measure was effective in isolation wards in hospital. The negative SARS-COV-2 RNA results of the objects surfaces such as door handles, PDA and gloves of staffs also suggested the effectiveness of the high hand hygiene compliance of staffs in isolation wards. Also, SARS-CoV-2 RNA was not detected among front surface of N95 masks of staffs in isolation wards. It also indicated that our staffs with PPE in the transformed isolation

wards with current disinfection measure were in low risk of infection. In this study all the respiratory specimens of staffs in the isolation wards were negative.

Since SARS-CoV-2 RNA was also detected in the stool specimen of patients with COVID-19 in previous reports (Yeo et al., 2020; Young et al., 2020), SARS-CoV-2 RNA was also detected in sewage in our study. For sufficient disinfection of SARS-CoV-2, preprocessing disinfection equipment were added before the sewage drained into the last disinfection pool. Though the sewage samples from the inlet of preprocessing disinfection equipment were positive for SARS-CoV-2 RNA and the sample from the outlet of preprocessing disinfection was weakly positive, the sewage sample from the final outlet of the last disinfection pool was negative. In order to identify the viability of the SARS-CoV-2, viral culture was done among these sewage from various points. All of the sewage samples from various points were negative for viral culture. This finding also indicated that the sewage drained from the hospital could not lead to transmission of the SARS-CoV-2.

This study also has several limitations. First, viral culture was not done among all of the 33 patients to demonstrate viability. Second, due to operational limitations during an outbreak, sample size was small. Third, the air was not sampled for SARS-CoV-2 RNA detection in the study. Further studies are required to confirm these preliminary results.

In conclusion, the SARS-CoV-2 RNA monitoring results of the hospital isolation wards demonstrated the routine disinfection measures of air, object surface and sewage in the hospital were sufficient and the hand hygiene of staffs was effective. Though the transmission of SARS-CoV-2 is associated with close contact to COVID-19 patients and droplet secretions of those patients, the strict disinfection and hand hygiene can decrease the hospital-associated COVID-19 infection risk of the staffs in isolation wards.

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Ethical approval

This study was approved by the local ethics committees of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Conflict of interest

The authors have no conflicts of interest to declare.

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