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Major Article

Evaluation of disinfection procedures in a designated hospital for COVID-19



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Key Words: SARS-CoV-2 Isolation wards Surfaces Sewage **Background:** Coronavirus disease 2019 has spread globally and been a public health emergency worldwide. It is important to reduce the risk of healthcare associated infections among the healthcare workers and patients. This study aimed to investigate the contamination of environment in isolation wards and sewage, and assess the quality of routine disinfection procedures in our hospital.

Methods: Routine disinfection procedures were performed 3-times a day in general isolation wards and 6-times a day in isolated ICU wards in our hospital. Environmental surface samples and sewage samples were collected for viral RNA detection. Severe acute respiratory syndrome coronavirus 2 RNA detection was performed with quantitative reverse transcription polymerase chain reaction.

Results: A total of 163 samples were collected from February 6 to April 4. Among 122 surface samples, 2 were positive for severe acute respiratory syndrome coronavirus 2 RNA detection. One was collected from the flush button of the toilet bowl, and the other was collected from a hand-basin. Although 10 of the sewage samples were positive for viral RNA detection, all positive samples were negative for viral culture.

Conclusion: These results revealed the routine disinfection procedures in our hospital were effective in reducing the potential risk of healthcare associated infection. Two surface samples were positive for viral detection, suggesting that more attention should be paid when disinfecting places easy to be ignored.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has globally spread and been declared as a pandemic by the World Health Organization on March 11, 2020. COVID-19 has been spreading fast because people are generally susceptible. The common symptoms of COVID-19 patients include fever, fatigue, dry cough, and the computed tomography scans showed bilateral patchy shadows or ground glass opacity in the lungs, while headache, dizziness, and gastrointestinal symptoms including abdominal pain, diarrhea, and vomiting are less common symptoms. The predicted incubation time of SARS-CoV-2 is 14days,

which may lead to a high rate of asymptomatic and subclinical infection among susceptible individuals, and people may get infected and be unaware of how or where it came from.³ Thus, effective interventions should be taken to break the chain of transmission of SARS-CoV-2.

Both confirmed patients and suspected individuals have potential to transmit the virus, since the viral shedding of asymptomatic patients has already been confirmed.^{4,5} The transmission of the virus is mainly by the respiratory droplets or by direct and indirect contact, and the viable SARS-CoV-2 particles could be detected for more than 1.5~3 hours in aerosols under experimental conditions.^{6,7} The droplets produced when COVID-19 patients coughed or sneezed could be transported over a long distance by airflow before settled on the surface, and the SARS-CoV-2 could stay viable for a long time after the droplets completely evaporated.^{8,9} The RNA purification is the gold standard for the detection of SARS-CoV-2 in samples, and the existence of SARS-CoV-2 RNA in the specimens of respiratory tract and feces of confirmed and asymptomatic patients have been confirmed

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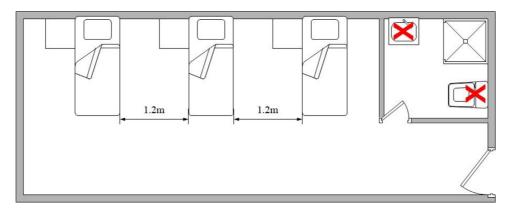


Fig 1. Room layout in isolation wards and location of positive surface samples for SARS-CoV-2 detection.
X: location of positive surface samples for SARS-CoV-2 detection.

by several researches.^{10,11} Therefore, the environmental contamination could be attributable to direct contact with infected respiratory fluids, feces and depositing of aerosol particles.¹² These results also indicate that sewage of the hospital could also be contaminated by COVID-19 patients' bodily fluids.

Many researches have reported the contamination of air and surfaces by SARS-CoV-2, emphasizing the importance of cleaning and disinfection in hospitals, despite the viability of the virus still need to be investigated.¹²⁻¹⁵ Due to lack of personal protective equipment (PPE), a lot of healthcare workers (HCWs) became infected during period of COVID-19 patients' care at the early stage of this pandemic.¹⁶ Thus, disinfection procedures are highlighted in mitigating healthcare associated infections (HAIs).

In this study, we enlarged the sample size and enhanced the sampling frequency to evaluate the quality of routine disinfection in isolation wards and sewage. In order to identify potential risk factors, places easy to be ignored were also sampled after disinfection, such as door handles, flush button of the toilet bowl, and hand-basin.

METHODS

Study design

This study was conducted in Zhijiang Campus, the First Affiliated Hospital of Zhejiang University, China. The hospital was originally a Grade 3A hospital and became one of designated hospitals for COVID-19 patients in Zhejiang province since the outbreak of COVID-19. The isolation wards were separated into 3 parts and 2 passages, including a contaminated area, a semicontaminated area, and a clean area. One passage was for patients, and the other was for medical stuff. The arrangement of the isolation wards was described in detail in the article we published before. ¹⁷ And the preprocessing disinfection equipment (Tianxingjian water-treatment Co., Ltd., Zhejiang, China) were added into the sewage disinfection system of the hospital. When compared with previous study, more places that could be potentially contaminated by COVID-19 patients were sampled for viral RNA detection, such as the flush button of the toilet bowl, medical refuse transfer trolley, elevators, and the examination rooms for these patients. Both respiratory and feces samples were collected during the hospitalization of COVID-19 patients and after their discharge to evaluate the quality of our disinfection procedures.

Patients with COVID-19 were confirmed by the positive polymerase chain reaction (PCR) results of respiratory samples. Both respiratory samples and feces samples were collected for PCR test during their hospitalization. Suspected individuals were hospitalized in single-person rooms with dedicated toilets. Confirmed patients were hospitalized into cohort-rooms with shared toilets in general isolation

wards or isolation intensive care unit (ICU) wards based on severity of illness. In a cohort-room, beds were spaced at least 1.2 meters apart, and up to 3 patients could be hospitalized in at a time (Fig 1).

Disinfection

Routine disinfection procedures were performed 3-times a day in general isolation wards and 6-times a day in isolated ICU wards. Continuously running plasma air disinfection machines (Model: PM-B1000Z2. Peijieer Medical Technology Co., Ltd., Zhejiang, China) were used for air disinfection in isolation wards, while the ultraviolet lights were used for air disinfection 3 times a day when no one was present if there were no plasma air equipment. Surfaces of objects and facilities were wiped with 1,000 mg/L chlorine containing disinfectant twice and waited for 30 minutes, then wiped with clean water. Visible contaminants including blood and other bodily fluids should be covered with wipes containing disinfectant (containing 5,000 mg/L chlorine) and completely removed before disinfection. Terminal disinfection was performed after the discharge of patients.

Sewages were disinfected in the preprocessing disinfection equipment treating with chlorine containing disinfectant before drained into the final sewage disinfection pool of the hospital. Disinfected sewages met the standard of Discharge standard of water pollutants from medical organizations (GB18466-2005).

Sampling

The environmental surface sampling was conducted in isolation wards where confirmed patients were hospitalized in. We collected samples from high-touch surfaces of the contaminated area, the semicontaminated area and the clean area in both general isolation wards and isolation ICU wards, including beeper, flush button, handbasin, door handle, bedside table, medical surface, elevator button, desktop, computer mouse and keyboard, telephone, and others. These samples were collected 2 hours after the routine cleaning and disinfection. We also collected samples from medical equipment and other high-touch surfaces in examination rooms for COVID-19 patients after terminal disinfection procedures.

ClassiqSwabs (Copan Flock Technologies, Brescia, Italy) were used for environmental surfaces sampling, and universal transport medium were used for sample transportation and storage before performing the PCR tests.

Sewage from the preprocessing disinfection equipment and the final disinfection pool were collected for viral RNA detection. Briefly, 1 mL sewage sample was centrifuged (12,000 \times g for 5 minutes at 4°C) to remove the solids, and 300 μL supernatant was collected for RNA extraction and PCR assay.

Quantitative reverse transcription PCR (qRT-PCR)

The MagNA Pure LC 2.0 (Roche, Basel, Switzerland) was used for sample RNA extraction. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay was used for SARS-CoV-2 detection, which was performed with a commercial kit (Biogerm Co., Ltd., Shanghai, China) approved by China Food and Drug Administration. The ORFab1 region and N gene on SARS-CoV-2 genome were employed for qRT-PCR assay, and the detection limitation was approximately 1,000 copies per mL. The cycle threshold value of amplification curve was defined as positive if less than or equal to 38.00.

Virus culture

To investigate the viability of virus in sewages, virus isolation and culturing was performed with Vero E6 cell line. All procedures were performed in a laboratory with qualified Biosafety Level 3. The samples were inoculated onto Vero E6 cell lines. After incubation for 96 hours, the cells were observed for cytopathic effect. Detection of viral nucleic acid in the culture medium indicates a successful culture.¹⁷

RESULTS

In this study, a total of 163 samples were collected from February 6 to April 4, of these: 105 environmental surface samples were collected in insolation wards during hospitalization of COVID-19 patients; 41 sewage samples were collected from the inlets or the outlets of preprocessing disinfection equipment; And 17 environmental surface samples were collected from the examination room after the terminal disinfection, including computed tomography room, endoscopy examination room, electroencephalogram examination room, and magnetic resonance imaging room.

Among 105 swab samples collected form environmental surfaces, 2 were positive for SARS-CoV-2 RNA (Fig 1, Table 1). One was collected from the flush button of the toilet bowl; the other was collected from a hand basin where a confirmed patient just spited the water used for gargling in. Both 2 positive sample were collected from a cohort room in general isolation ward with 3 patients hospitalized in. All respiratory and feces samples of these patients were positive when environmental surface samples were collected, despite only 1 patient had gastrointestinal symptoms.

Of the paired sewage samples collected from the preprocessing disinfection equipment, 6 (6/14) from the inlets and 4 (4/14) from the outlets were found to be positive for SARS-CoV-2 RNA testing (Table 2). The viral culture of these samples was negative. All samples collected from the final disinfection pool were negative for SARS-CoV-2 RNA testing.

All samples collected from the examination rooms after the terminal disinfection were negative for SARS-CoV-2 RNA testing (Table 3).

DISCUSSION

It was reported that the COVID-19 has caused more than 82,341 infections across China, over 3,000 of which were in HCWs by April 15, 2020.¹⁶ Most of the infected HCWs were confirmed at the early stage of this pandemic, when environmental cleaning, and

Table 1SARS-cov-2 detections in the surface samples collected from the isolation wards in the interval between routine disinfection procedures

Areas	Sampling sites	Total number of samples	Positive number of samples	Cycle threshold value
Contaminated	Beepers	2	0	
area	Flush button of the toilet bowl	17	1	36.53
	Hand-basin	9	1	36.83
	Bedrails	2	0	
	Bedside tables	16	0	
	Door handles	9	0	
	Telephones	2	0	
	Medical equipment	7	0	
	Desktops	5	0	
	Medical refuse transfer trolley	6	0	
	Nurse station	10	0	
	Chairs	3	0	
	Computer mouses	3	0	
	Nursing trolley	5	0	
Semi-contaminated	Computer mouses	2	0	
area	Keyboards	2	0	
Clean area	Elevator hall	2	0	
	Elevators	3	0	

disinfection protocols were not established and HCWs were not aware of the potential risk of getting infected.¹⁸

A research of the stability of SARS-CoV-2 indicated that the virus could be detected in 4 hours on copper and 24 hours on cardboard, while it could be detected up to 72 hours after application to plastic and stainless steel. Despite of the human-to-human transmission, the contact of contaminated surfaces may contribute to the HAIs among the HCWs and patients. Herefore, the environmental cleaning and disinfection protocols were extremely important in preventing the HAIs.

All Covid-19 patients and suspected individuals without mechanical ventilation were wearing surgical masks during their hospitalization in our hospital. And alcohol-based hand hygiene sanitizer dispensers were placed nearby the beds in the patient's room. Since the environment could become contaminated with the virus, touching infected patients or patient surroundings could be a possible route for SARS-CoV-2 transmission, which would eventually cause the HAIs among patients and HCWs. It was important to perform the hand hygiene to stop the spread of the virus from 1 place to another. In our hospital, PPE was used by HCWs in isolation wards, including N95 masks, gloves, and disposable isolation gowns. The PPE was removed in the removal area, thus HCWs were required to perform hand hygiene without removing gloves in isolation wards. The key moments and procedures for hand hygiene were in accordance with the World Health Organization guidelines. 19 Combined with routine disinfection, these procedures were proved to be effective according to the negative results of the samples collected from the high-touch surfaces in the isolation wards.

Both of the positive samples were collected in the toilet of confirmed patient's room. The patients who stayed in were positive in both respiratory samples and feces samples. When patients with

Table 2SARS-cov-2 detections in the sewage samples collected from preprocessing disinfection equipment and final disinfection pool

Sites	Total number of samples	Positive numbers of samples	Cycle threshold value
Inlets of preprocessing disinfection equipment	14	6	35.97, 37.80, 28.78, 30.58, 29.37, 35.93
Outlets of preprocessing disinfection pool	14	4	27.72, 33.55, 34.35, 34.58
Final pool of sewage disinfection	13	0	

Table 3SARS-cov-2 detections in the surface samples collected from the examination rooms after the terminal disinfection

Sites	Total number of samples	Positive numbers of samples
Endoscopy room	4	0
Computed tomography room	6	0
Electroencephalogram Examination room	5	0
Magnetic resonance imaging room	2	0

COVID-19 brushing their teeth or gargling, the virus in the secretions from the respiratory mixed with the water would be spited into the hand basin. So, the viral RNA could be detected from the samples collected from the hand-basin, and the contamination of hand basin could attributable to the direct contact with respiratory fluids. Usually, patients would flash the toilet after using first, before washing their hands. Therefore, viral RNA was detected on the toilet flush button, and the contamination of flush button could attributable to the direct contact with feces. These results indicated the surfaces in isolation wards could be contaminated by respiratory secretions, feces, or other bodily fluid of infected patients. It could cause the transmission of virus and cross infection among patients and HCWs. Similarly, other places that were easy to be ignored could also be contaminated, such as toilet seat and elevator buttons, in spite of the viral RNA were not detected from the surface samples of these places in this study. More attentions were needed in daily disinfection and terminal disinfection when cleaning these places.

When patients with COVID-19 needed CT or electrocardiogram examinations, it should be performed in the specific examination rooms. Patient with COVID-19 should be scheduled as the last patient of the day. The terminal disinfection was needed after all examinations for confirmed patients were completed. When suspected individuals needed the examinations, the rooms should be disinfected every time after 1 patient had completed his examination to prevent the cross infections.

As the epidemic had been brought under control in China, few new cases were found in Zhejiang province, and patients with COVID-19 were recovered and discharged from our hospital. Terminal disinfection and environmental sampling was required for the isolation wards and examination rooms in order to provide medical services for non-COVID-19 patients. The disinfection procedures for examination rooms were consistent with the procedures for the isolation wards. These areas could not be used for non-COVID-19 patients until all the environmental samples collected were negative for SARS-CoV-2 RNA detection. In this study, surface samples collected from the examination rooms were all negative for SARS-CoV-2 RNA detection, and the samples collected from isolation wards and other places were also negative for viral RNA detection, which indicated that the terminal disinfection was effective.

The presence of SARS-CoV-2 RNA in feces of COVID-19 patients had been widely reported, ²⁰⁻²² which meant the virus could also be detected in the sewage. It had been verified by some researches about the wastewater in USA, France, Italy, and China. ^{17,23-25} Individuals could get infected with sewage which contained the SARS-CoV-2 virus via the fecal-oral route, in spite of the viability of SARS-CoV-2 virus in sewage was still unknown. ²⁶ To our knowledge, few studies have provided insights into whether the contaminated sewage could cause COVID-19. ²⁷

In order to sufficient inactivate SARS-CoV-2 virus in the sewage drained into urban sewage, the preprocessing disinfection equipment were added into the sewage disinfection system of the hospital. Although some sewage samples collected from the inlets and outlets were positive, the results of viral culture for these samples were all negative. This result indicated there was no viable virus due to

disinfection in the preprocessing disinfection equipment. And all samples from the final disinfection pool were negative. These results indicated the disinfection of patients' feces was necessary to reduce the potential risk of SARS-CoV-2 transmission.

Our study had several limitations. First, we did not collect the air samples due to the lack of air sampling machine. Other researches had revealed the presence of SARS-CoV-2 RNA in aerosol, which indicated the air could be contaminated by the virus, and patients could be infected in the isolation wards. Second, the positive samples of surfaces were determined by PCR tests, while the ability of virus could not be determined. Third, we collected the samples at single time point in the disease process of individual patients, and did not investigate whether there is any relationship between the levels of environmental contamination and the disease.

In conclusion, during the COVID-19 pandemic, our hospital implemented a set of disinfection procedures to reduce the potential risk of HAIs via the directly contact of contaminated surfaces. The environmental surveillance of SARS-CoV-2 in this study revealed our routine disinfection procedures were effective, despite 2 samples tested positive for SARS-CoV-2 RNA. No HAI of COVID-19 were detected in our hospital. Additionally, the disinfection of patients' feces was sufficient for the inactivation of SARS-CoV-2 in the sewage drained from the hospital. These findings indicated the disinfection procedures performed in our hospital were effective in preventing the HCWs from getting infected. Further studies with larger sampling size were required to clarify the relationship between the levels of contamination and the disease.

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