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The inert surface contamination of SARS-CoV-2 and the effect of disinfectants in one of the specialized and main responsible hospitals for COVID-19 patients in Ahvaz, Iran

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Abstract

Background: SARS-CoV-2, the causative agent of the COVID-19 pandemic, spreads through both direct and indirect pathways. Among the latter, surface contamination is a significant concern due to the virus's prolonged viability on surfaces. There is ongoing discussion over the impact of environmental surface contamination, especially in light of the introduction of novel viral types. The present study aimed to examine the extent of environmental surface contamination across different hospital wards and evaluate the effectiveness of disinfectants in inactivating the virus.

Methods: The samples were collected from critical areas in a hospital, both pre-disinfection (n = 40) and post-disinfection (n = 17), using reverse transcription-polymerase chain reaction (RT-PCR) to detect SARS-CoV-2.

Results: The findings indisputably confirm the presence of SARS-CoV-2 on swab samples from frequently-touched surfaces. Notably, 10 samples were virus-positive before disinfection, highlighting persistent viral contamination in vital hospital zones.

Conclusion: This study underscores the critical role of environmental surface contamination in SARS-CoV-2 transmission, particularly in healthcare settings. Detecting the virus on frequently-handled surfaces underscores the urgent need for rigorous and frequent surface disinfection. Effective surface disinfection remains a rapid, straightforward, and practical strategy to mitigate virus transmission to healthcare workers and patients. These findings hold significant implications for infection control, particularly amid emerging virus variants. They emphasize the need to maintain stringent hygiene and disinfection practices within healthcare facilities to combat the spread of COVID-19.

Keywords: SARS-CoV-2, COVID-19, Disinfection, Virus inactivation, Humans

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Introduction

Wuhan, China, reported several pneumonia-related incidents in December 2019. This causes a respiratory illness that was reported as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the World Health Organization (WHO) (1-3). The WHO expressed the outbreak of a Public Health Emergency of International Concern on January 30, 2020 (4-6).

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There are different mechanisms and pathways for the transmission of this disease. These pathways include direct pathways, which involve direct contact with an infected person, and indirect pathways, which involve contact with contaminated environmental surfaces and foods, as well as airborne transmission through viral infectivity (7-10). Fomites are objects contaminated with infectious agents (case, SARS-CoV-2) and support their transmission. Hence, the transmission of this virus mostly occurs through two important routes including direct contact with an infected individual and indirect contact with contaminated surfaces and objects (11-13). The SARS-CoV-2 virus was shown in numerous experiments to be highly contagious for hours in aerosol form and for days on surfaces (14-17). The consistency of SARS-CoV-2 was similar to that of SARS-CoV-1 under the experimental situations trialed (18,19). Broadly speaking, SARS-CoV-2, similar to other human coronaviruses, can remain infectious on dry surfaces at room temperature for a considerable amount of time - ranging from hours to days. Nevertheless, it has been proven that the virus can be efficiently inactivated through surface disinfection (20). To mitigate the possible transmission of SARS-CoV-2 from surfaces, the WHO suggests the utilization of water, detergents, and disinfectants as effective means to decontaminate surfaces and create a more sanitized environment (16,21,22). Unfortunately, hospitalized individuals with high viral loads in their respiratory tract facilitate the transmission of COVID-19 within the hospital setting through the dispersion of droplets when coughing or sneezing (23). In confined spaces, droplets can remain suspended for more than 10 min, making it possible for disease transmission over significant distances (24,25). Razi Hospital was the reference hospital for COVID-19 patients in Ahvaz, the center of Khuzestan province, during the COVID-19 pandemic. This study aimed to investigate the contamination of the surfaces by coronavirus as well as the durability of the virus to disinfectants, in the COVID-19 section of Razi Hospital to identify potential risk factors and potential for environmental pollution by patients with COVID-19, and finally, to perform healthcare protocols and disinfection frequency of critical points properly.

Materials and Methods

Sampling site description and procedures applied

The present study was conducted on November 15, 16, 2020, and December 20, 2020, during the second wave of COVID-19, in the COVID-19 Ward of Razi Hospital in Ahvaz, the center of Khuzestan province in southwest Iran as presented in Figure 1.

Surfaces and objects were wiped down daily with Alcohol (ethanol 70%) disinfectant. The sampling was carried out before and one minute after the disinfection operations. During sampling, the temperature and relative humidity (RH) of the COVID-19 Ward ranged from 32-35 °C and 33%-42%, respectively, while the average outdoor temperature and RH in Ahvaz at the same time were 20 to 22 °C and 50-55%, respectively. In the present research, swab (n = 57) samples were collected before (n = 40) and after (n = 17) disinfection from 20 critical zones. The study was performed in different wards of the hospital including Isolation, Infection, Emergency, and nursing Wards with 174 patients whose test was positive for SARS-CoV-2. Sampling was performed from different surfaces of the hospital wards including staff water cooler, staff gloves, inside of the mask used by staff, nursing station, staff dining table, ventilator mask, suction machine, medical recorder (metal), patients' cellphone, staff cellphone, isolation-patient locker, infection-door handles, patients' oxygen saving mask, isolation-patients' toilet tap, and soapbox, patients' console, emergency-phone, isolation-bed rail emergency-laundry room, isolation-oxygen manometer, isolation patients toilet, and infection-bed rail The WHO guidelines were weighed for sampling (26). Considering sterile conditions, surface samples were collected using a swab. The swab was taken away from the package, and wet to the viral transport medium. After swabbing a 10 cm^2 area with adequate pressure and rotating the swab stick for 30 seconds, the swab was inserted into sterile falcon tubes containing viral transport medium in a seamless bag. To obtain better results and more accurate sampling, three swaps were used for each surface, and all three swaps related to one surface were placed inside a falcon tube containing the culture medium. Before being placed in the transport container, the seamless bag underwent cleaning using a 70% ethanol solution. Control samples were collected using the same methodology as the environmental samples collected from the possibly contaminated region; this involved opening the package and extracting the swab from the tube but refrained from sampling any surfaces (27). All samples were transferred to the laboratory with a cool box at a temperature of around 4°C (16,28).

Laboratory analysis

The virus genome was extracted using the SinaPure Viral kit (Sinaclon, Iran) and stored at -70 °C. The purity of the extracted RNAs was assessed using a Nanodrop spectrophotometer (Thermo Scientific, USA). The virus was confirmed in the laboratory using real-time reverse transcription-polymerase chain reaction (RT-PCR). As expressed by the instructions of kit (Pishtaz Teb Zaman, Iran), a certain amount of extracted RNA (5 μL) was poured into One-Step qRT-PCR master mix (15 μL) supplemented with a mixture of primers and TaqMan probes directed against RdRp and N genes. Amplifications and further analysis were conducted using the Applied Biosystems Step One Plus RT-PCR System. To conduct thermal cycling, we began with reverse transcription at 50

Figure 1. The study area (Razi Hospital in Ahvaz, the center of Khuzestan, Iran)

°C for 20 minutes. Next, we initiated denaturation at 95 °C for 3 minutes, followed by 45 cycles at 94 °C for 10 seconds and 55 °C for 40 seconds. The samples with cycle threshold (Ct) values less than 40 cycles were considered positive. In case the Ct values were between 40 and 45 cycles, the PCR test was repeated. Negative control samples were included in each experiment to identify any contamination that may occur. A positive control sample was also included in every experiment to detect any false-negative results (7).

Results

In this study, the quantity and quality of SARS-CoV-2 were assessed in different wards of Razi Hospital in Ahvaz, one of the leading centers for providing medical services to patients with COVID-19 infection in the province, in two stages before and one minute after disinfection. The samples were taken from repeatedly-touched surfaces in the hospital. SARS-CoV-2 RNA was detected on various surfaces in different hospital wards where outbreaks have occurred. Table 1 presents the laboratory test results before disinfection. Furthermore, a comparison between the number of positive cases and cases sampled in positive samples was presented in Figure 2. As depicted by Table 1, the samples were collected from contaminated environmental surfaces in various hospital wards including staff water coolers, staff gloves, inside of the mask used by staff, nursing stations, staff dining tables, ventilator masks, suction machines, medical recorders (metal), patients' cellphone, staff' cellphone, isolation-patient locker, infection- door handles, patient' oxygen saving mask, isolation-patients' toilet tap and soapbox, patients' console, emergency-phone, isolation-bed rail emergency-laundry room, isolation- oxygen manometer,

Table 1. RT-PCR results of various wards of the hospital's surface samples before disinfection

Sampling sites	Result		Number of samples
	Positive	Negative	
Staff's water cooler	0	1	1
Staff's gloves	2	1	3
Inside of the mask used by staff	1	1	2
Nursing station	0	4	4
Staff's dining table	0	2	2
Ventilator mask	1	1	2
Suction machine	1	0	1
Medical recorder (metal)	1	1	2
Patients' cellphone	0	2	2
Staff' cellphone	1	1	2
Isolation-patient locker	0	2	2
Infection-door handles	1	1	2
Patient's oxygen-saving mask	1	1	2
Isolation-patient's toilet tap and soapbox	0	1	2
Patient' console	0	2	2
Emergency-phone	0	2	2
Isolation-bed railing	1	2	3
Emergency-laundry room	0	1	1
Isolation-oxygen manometer	0	2	2
Isolation-patient's toilet	0	1	1

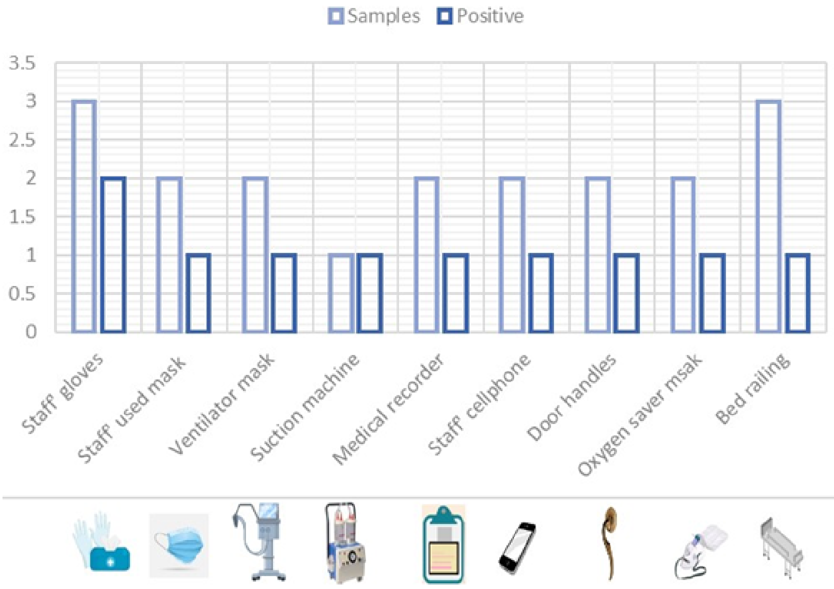

Figure 2. Comparison between the number of positive cases and cases sampled in positive samples

isolation patients toilet, infection-bed rail sampling and PCR results related before disinfection showed that out of 40 samples, 10 cases (25%) were positive, which were mainly related to close contact with the patients. Table 2 shows the environmental conditions of surface sampling in different hospital wards. Temperature (°C) and relative humidity (RH%) were recorded in the intended wards of the hospital during the sampling period; the recorded values were between 32 and 35 °C and 33%–42%, respectively. Table 3 the laboratory test results after disinfection.

Table 2. Environmental conditions of surface sampling in various wards of the hospital (status of windows: close)

Sampling sites	Ventilation system	Humidity (%)	Temperature (°C)
Staff's water cooler	Ventilation off	33	32
Staff 'gloves	Ventilation off	34	33
Inside of the mask used by staff	Ventilation off	38	34
Nursing station	Ventilation off	35	33
Staff's dining table	Ventilation off	35	34
Ventilator mask	Ventilation off	36	35
Suction machine	Ventilation off	38	35
Medical recorder (metal)	Ventilation off	35	34
Patient' cellphone	Ventilation off	36	35
Staff' cellphone	Ventilation off	36	35
Patient locker	Ventilation off	37	34
Door handles	Ventilation off	35	34
Patient's saving mask	Ventilation off	38	35
Patient's toilet tap and soapbox	Ventilation off	40	35
Patient' console	Ventilation off	38	34
Emergency-phone	Ventilation off	38	35
Emergency-bed railing	Ventilation off	38	35
Emergency-laundry room	Ventilation off	42	34
Isolation-oxygen manometer	Ventilation off	41	35
Isolation-patients' toilet	Ventilation off	42	35

Discussion

The spread of COVID-19 is presumable to attenuate at relatively high temperatures and humidity (29). Many research has corroborated that viruses fall more quickly at low temperatures and high humidity and they can spread as droplets or aerosols, which preserve large particle sizes and are heavier at high humidity, and thus, can settle rapidly or be distracted by masks, nasal cavity, etc.; finally, high temperature and high humidity improve human immunity (30-33). Studies have revealed that viable SARS-CoV-2 can persist on commonly touched surfaces for different periods, depending on the surface material (stainless steel up to 72 hours 5-6, cardboard/paper up to 24 hours 3-4, plastic up to 72 hours 6-7, copper up to 4 hours ≈1, at room temperature) and environmental characteristics (34,35). SARS-CoV-2 can be viable on the surface longer than other coronaviruses (36,37). Since the high transmissibility has been confirmed for SARS-CoV-2, it is crucial to investigate environmental surfaces for COVID-19 animated transmission risk. After disinfection, there were no positive cases among the 17 samples. Ge et al. in their study expressed that 105 swab samples were gathered from hospital environmental surfaces in which disinfection proceeded with 1000 mg/L chlorine. Only two samples were positive for SARS-CoV-2 RNA; they were collected from the flush button of the toilet bowl and hand basin of the confirmed COVID-19 patient room (38). Some studies demonstrated the capability of SARS-CoV-2 to maintain inanimate surfaces against

Table 3. RT-PCR results of various wards of the hospital's surface samples after disinfection

Sampling Sites	Number of samples	Result
Nursing station	4	Neg
Staff's water cooler	2	Neg
Staff's dining table	1	Neg
Emergency-phone	2	Neg
Infection-bed railing	2	Neg
Isolation-patient locker	2	Neg
Isolation-patient's toilet taps and soapbox	2	Neg
Infection-door handles	2	Neg

used disinfectants (7,25,39). Noorimotlagh et al in their investigation collected 76 samples from different wards of the hospital after disinfection with ethanol 70% and sodium hypochlorite (0.001%), of which 40 were positive. Furthermore, it can be considered that the level of viral load of patients or infected persons in the hospital has an important role in the survival of SARS-CoV-2 despite the disinfection proceed (7,40,41). The findings of the present study show that the environment of COVID-19 patients is widely contaminated, and the staff of health centers is constantly in contact with contaminated surfaces, which significantly increases the eventuality of transmission. This study showed that only the surface in close contact with the COVID-19 patient was positive for SARS-CoV-2. Negative results on the surfaces associated with the medical staff, such as telephone, nursing station, staff dining table, and staff locker room water cooler, indicate that the health protocols are well observed by the medical staff at this hospital. Also, the positive results in the fomites that are in contact with the patients show that the virus is always transmitted to the environment by the patients.

Conclusion

This study was conducted in the COVID-19 Ward of the Razi hospital in Ahvaz, the center of Khuzestan province in southwest Iran. A total of 57 samples were collected before (n = 40) and after (n = 17) disinfection from 20 critical zones. The PCR results of samples after disinfection showed that disinfection there were not any positive cases among the 17 samples. These data suggest that the hospital surface contamination was very severe before disinfection, particularly when environmental cleaning regulations were not followed; this poses a significant occupational hazard for healthcare staff, patients, and visitors, highlighting the importance of maintaining proper hygiene practices in healthcare settings. Since COVID-19 spreads by droplet infection, its transmission speed may be higher than other infectious viruses. Thus, the quickest, most effortless, and most practical way to suspend or decrease the reach of SARS-CoV-2 outcome in this disease is via surface disinfection and regard for environmental hygiene tenets. In this regard, disinfection of different hospital wards should be done according to the instructions of environmental health and infection control. It is recommended that according to the CDC and WHO guidelines, disinfection should be done regularly before and after work shifts and at least three times a day. Service personnel responsible for disinfection and infection control must be continuously trained in the implementation of instructions and must be fully aware of the performance of their duties.

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Competing interests

The authors declare that there are no conflict of interests.

Ethical issues

The authors hereby certify that all data collected in the field of study were described in the manuscript and no data from the study have been or will be published elsewhere separately (Approval code: 122, Ethical code: IR.AJUMS.REC.1399.149).

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