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Coronavirus Pandemic

Role of hospital environmental surfaces in the transmission of the severe acute respiratory syndrome - Coronavirus-2

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Abstract

Introduction: Severe acute respiratory syndrome - Coronavirus-2 (SARS-CoV-2) is mainly transmitted via respiratory secretions through coughing, sneezing, or contact with contaminated surfaces. This virus can be present in feces and many body fluids. The study aimed to screen the hospital environment as a potential source for SARS-CoV-2 transmission and identify the hospital zones with the highest contamination levels.

Methodology: Swabs were collected from different sites in the hospital before and after routine cleaning/disinfection, transported in vials containing 1-3 mL of viral transport medium, and stored at -80 °C as soon as possible until the time of testing. The real-time reverse-transcription PCR (rRT-PCR) system targeting *RNA-dependent RNA polymerase* and *E* genes was used to detect the SARS-CoV-2 RNA.

Results: Moderate environmental contamination by SARS-CoV-2 RNA was detected by rRT-PCR before routine cleaning/disinfection (52% of the swabs were positive). The hospital surfaces with the highest contamination levels were elevators' buttons, sinks and faucets' handles at the waiting rooms, patient's room and bathroom, call buttons and telephones in the patient's room, toilet bowl surface, the doorknob and light switches at the X-ray room, and the computer keyboard at the staffroom. All the swabs collected after routine cleaning/disinfection were negative for SARS-CoV-2 RNA by rRT-PCR.

Conclusions: The hospital environment is a high-risk area that can be contaminated by SARS-CoV-2 through contact, respiratory, and maybe fecal shedding of the virus. To limit this fatal virus transmission, strict adherence to proper hand hygiene with frequent optimal decontamination of hospital environmental surfaces is essential.

Key words: Contamination; COVID-19; decontamination; SARS-CoV-2; swabs.

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Introduction

By the end of December 2019, several cases of pneumonia were identified in Wuhan city, China. These cases of pneumonia presented with several symptoms ranging from symptomatic to variable clinical manifestations like dry cough, dyspnea, fever, respiratory distress, or respiratory failure [1]. Upon detection of these cases, there was a link found between the index cases on one hand and seafood markets and wildlife on the other hand [2].

The causative virus was detected by the center for disease and control in China (CCDC) from throat swabs samples on 7th January 2020 [3]. The severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) is the causative pathogen of the rapidly spreading fatal Coronavirus Disease-2019 (COVID-19) [4]. Coronaviruses belong to a family known as *Coronaviridae* and are among the largest single-stranded, positive-sense, enveloped RNA viruses with a genome size of 30 Kb [5]. Although SARS-CoV-2 is very sensitive to heat, it is highly stable at 4 °C and stable in a wide range of pH at room temperature [6].

SARS-CoV-2 mainly spreads through respiratory secretions via coughing, sneezing, or contact with contaminated surfaces. There is a potential risk for SARS-CoV-2 shedding in feces and other body fluids [7-9]. Its incubation period ranges between two to fourteen days [10]. Unfortunately, until now there is no approved antiviral therapy for COVID-19 treatment. Vaccination and applying strict infection prevention and control measures are the main ways to limit this viral spread [11].

Hospitals could be significant centres for the transmission of infection for patients, healthcare workers (HCWs), and visitors [12]. Nearly half a century ago, Mahl and Sadler (1975) reported the persistence of multiple viruses on many inanimate surfaces and suggested, for the first time, the possibility of virus transmission via inanimate surfaces [13]. Later on, information on the possible role of contaminated inanimate surfaces in the transmission of viruses (including the SARS-CoV that emerged in 2002) has amplified quickly and many researchers concluded that most respiratory tract viruses can persist on inanimate surfaces for a few days with the possibility of their transmission through contaminated surfaces if optimal preventive surface cleaning/disinfection are not performed [14-16]. MERS-CoV and SARS-CoV are very sensitive to detergents/disinfectants [17].

The contaminated hospital environment is a potential high-risk area that is suspected to facilitate the SARS-CoV-2 spread. There is limited data about the role of the hospital environment in the transmission of SARS-CoV-2. In this research, we tried to understand the role of the hospital environment in the transmission of this fatal virus and to identify the hospital zones with the highest contamination levels.

Methodology

Sampling Technique

Environmental Sample sites

According to the Ministry of Health and Population, Tami Al-Ameed Central Hospital, is a large hospital in Dakahlia-Egypt that serves thousands of inpatients, outpatients and medical emergencies, in addition during the current COVID-19 pandemic it was considered as a central hospital for isolation and treatment of Coronavirus. Twenty-five surface hospital environmental sites, including the personal protective equipment (PPEs) of the medical staff, were swabbed six times, four of them before (with one-week interval) and two after routine cleaning/disinfection (Table 1).

Environmental sampling method

After wearing the standard PPEs including sterile gloves, 150 environmental surfaces were swabbed at least in two directions by using Dacron swabs wetted by the viral transport media (100 before and 50 after routine cleaning/disinfection) [18,19].

Labeling of the samples

The date, time, location of sampling, transportation conditions, and the time of arrival at the laboratory were recorded. After labelling, the vial was put in a sealing bag and the outside was disinfected with 70% ethanol solution.

Transportation of the samples

The swabs were collected in vials containing 1.2 mL of viral transport medium (VTM) that contained antibiotics, protein stabilizer, and neutralizing buffer that counteracts any residual effects of the previously used disinfectants (e.g., Tween 80).

Storage of samples

The samples were stored at -80 ℃ as soon as possible until testing by real-time reverse-transcription-polymerase chain reaction (rRT-PCR) within a maximum limit of three days.

Control samples

Control negative swabs were included by opening the package and removing the swab from the tube but without sampling any surfaces.

RNA extraction

According to the instructions of the RNA extraction kit (QIAamp® Viral RNA Mini Kit, QIAGEN®, Hilden, Germany), RNA was extracted from the samples. If in time PCR testing is not possible, the extracted RNA templates were stored at -80℃.

Quantitative real-time RT-PCR

The detection of the SARS-CoV-2 *RNA-dependent RNA polymerase* (*RdRp*), and *envelope protein* (*E*) genes was done by using a specific kit (1 copy™ COVID-19 qPCR Multi-Kit, Gyeonggi-do, Republic of Korea). The amplification was based on real-time fluorescent PCR technology using specific primers and probes in the rRT-PCR system. In the PCR reaction, the Taq DNA polymerase 5' nuclease activity resulted in TaqMan probe degradation, reporter dye separation from the quencher with the generation of fluorescent signals that were monitored during every PCR cycle by the PCR system as the following; in the first well, there was FAM channel qualitative detection of SARS-CoV-2 *E* gene in *E* gene assay mixture and in the second well, there was qualitative detection of SARS-CoV-2 *RdRp* gene in *RdRp* gene assay mixture. Control 1 (*E* gene plasmid) and control 2 (*RdRp* gene plasmid) were used as positive controls. Texas Red channel detection of internal positive control (human *GAPDH* gene) was used. Negative control was included in the reaction (DW NTC; no template control). Contamination of the PCR amplification products was avoided by including UNG and dUTP enzymes in the reaction. Thermal

cycling conditions were reverse transcription for 10 minutes at 55 °C followed by initial denaturation for 3 minutes at 95 °C and 45 cycles of amplification for 15 seconds at 95 °C and 30 seconds at 58 °C as mentioned before [19]. The viral load was quantified from the cycle threshold value (CT-value) which is the number of cycles required for fluorescent signals to cross the rRT-PCR threshold (lower cycle threshold values indicate higher viral loads). A CT-value > 40 or more was considered an invalid (negative) test, and a CT-value ≤ 40 was considered a valid (positive) test.

Data analysis

The percentage of positivity was calculated for the hospital environmental sites with positive swabs.

Ethics Statement

Bioethical approval (number 10-08/42) was obtained from the local committee of bioethics of Jouf University, Saudi Arabia.

Results

There was moderate environmental contamination by SARS-CoV-2 RNA before routine cleaning / disinfection. SARS-CoV-2 rRT-PCR was positive for 52 out of 100 (52%) swabs collected before routine cleaning/disinfection as described in Table 1. Positive results are shown as the number of positive samples/ (4 which is the number of total samples taken before routine cleaning/disinfection). The hospital surfaces with the highest contamination levels were elevators' buttons, sinks and faucets' handles in the waiting rooms, patient's room and bathroom, call button and telephone in the patient's room, toilet bowl surface, the doorknob and light switch at the X-ray room, and the computer keyboard at the staffroom. All (n = 50, 100 %) the swabs collected after routine cleaning/disinfection were negative for SARS-CoV-2 RNA by rRT-PCR.

Discussion

Little data are available about SARS-CoV-2 transmission through contaminated hospital environmental surfaces. In the current study, contamination of hospital environmental surfaces by SARS-CoV-2 RNA was detected (52% of the swabs collected before routine cleaning/disinfection were

Table 1. Sampled environmental sites and the corresponding real-time reverse-transcription PCR (rRT-PCR) results.

Possible sites of hospital transmission	Sampling Sites [a]			Positive samples (Before routine cleaning) [b]	Cycle threshold value [c]
Patients' entry	Ambulance	1.	Medical bag handles.	2/4	35.79, 37.54
		2.	Ceiling.	0/4	
	Entrance of hospital	3.	Doorknob and Light switch.	2/4	35.79, 35.79
		4.	Sink and faucet handles.	2/4	35.79, 37.54
	Elevators	5.	Buttons.	4/4	35.79, 35.83, 36.79, 37.54
	Waiting rooms	6.	Doorknob and Light switch.	2/4	35.83, 37.75
		7.	Sink and faucet handle.	4/4	35.54, 35.79, 35.83, 37.94
Patient handling and care	Patient's room	8.	Doorknob and Light switch.	2/4	37.07, 37.94
		9.	Sink and faucet handle.	4/4	32.96, 35.83, 35.83, 37.94
		10.	Bed rails and bed controllers.	2/4	35.79, 35.83
		11.	Bedside table.	0/4	
		12.	Call button and telephone.	4/4	30.64, 35.79, 35.79, 35.83
		13.	Floor.	2/4	30.64, 35.79
		14.	Chair and curtain.	2/4	34.89, 35.79
		15.	Clothes.	2/4	35.79, 37.11
	Bathroom	16.	Doorknob and Light switch.	2/4	35.79, 36.21
		17.	Sink and faucet handle.	4/4	37.54, 37.94, 37.54, 38.96
		18.	Toilet bowl surface.	4/4	35.71, 37.54, 36.21, 37.54
	X-ray room	19.	Doorknob and Light switch	4/4	35.44, 37.95, 37.54, 36.21
		20.	Sink and faucet handles	0/4	
		21.	X-ray table	0/4	
Medical staffs' room	Staffroom	22.	Doorknob and Light switch	0/4	
		23.	Computer keyboard	4/4	35.54, 36.21, 36.21, 37.54
		24.	PPEs	0/4	
	Anteroom	25.	Doorknob and Light switch	0/4	

[a] Six swabs were collected from each site, four of them before (with one-week interval) and two after routine cleaning and disinfection. All the swabs collected after routine cleaning and disinfection were negative and were not included in the table); [b] Results are shown as the number of positive samples/number of total samples taken before routine cleaning; [c] Cycle threshold refers to the number of cycles required for the fluorescent signal to cross the threshold in rRT-PCR; a lower cycle threshold value indicates a higher viral load; rRT-PCR, real-time reverse-transcription PCR; PPEs, personal protective equipments.

positive) by rRT-PCR as described in Table 1. This result is in agreement with many studies [16,20,21].

Some studies reported lower levels of hospital environmental surface contamination. An Italian study reported a 7.7% environmental surfaces contamination rate (2 of 26 samples were positive) with very low viral loads. The Italian study assessed the viral viability using susceptible (Vero E6) cells, but no cytopathic effect was detected after seven days of viral culture [22]. Another study from China reported a 25% environmental surfaces contamination rate (50 out of 200 samples were positive) with the top five positive sampling sites being water machine buttons, beepers, elevator buttons, telephones, and computer mouses but the study did not assess the viral viability [23].

In the conducted study, most (16 out of 28; 57.1%) swabs collected from the patients' entry area before routine cleaning/disinfection were SARS-CoV-2 RNA positive by rRT-PCR including medical bag handles, doorknobs, light switches, sinks, faucet handles, and elevators' buttons. The highest contamination levels in this hospital zone were detected in the elevators' buttons, sinks, and faucets' handles in the waiting rooms. This may be explained by their frequent handling with contaminated hands or gloves.

Similarly, widespread SARS-CoV-2 RNA contaminations outside the infected patients' rooms have been reported [18,24]. Gonçalves and his research team [25] did a systematic review that summarized the published studies that detected SARS-CoV-2 RNA on inanimate surfaces by using molecular methods (till July 2021). The systematic review included 37 eligible studies and the authors reported that contamination of the surfaces with SARS-CoV-2 RNA was detected on a wide range of surfaces with the highest contamination rates were detected in healthcare facilities where 17.7% and 10.1% of the samples that were taken from hospital settings and non-hospital settings were positive, respectively. Furthermore, in their systematic review, they found that only six [22,24,26-29] out of the 37 eligible studies have tested the viability/infectivity of the detected SARS-CoV-2 RNA from 242 positive surface samples but the viral viability was not confirmed. Although studies of SARS-CoV-2 viability on surfaces are scarce, surface transmission may be possible [30].

Regarding the patient's room in the performed study, most (18 out of 32; 56.3%) swabs taken before routine cleaning/disinfection were positive for SARS-CoV-2 RNA by rRT-PCR including doorknob, Light switch, sink, faucet handles, bed rails, bed controllers, call button, telephone, floor, chair, curtain, and clothes. The highest contamination levels at this hospital zone were detected in the sink, faucet handles, call buttons, and telephone. This was expected and agrees with the results of many studies with variable contamination levels. A study performed in Hong Kong, China reported a 5% environmental surfaces contamination rate at the patients' areas with the highest contaminations on patients' mobile phones, followed by bed rails and toilet door handles but the study did not assess the viral viability [31]. Another study was performed by the Nebraska Medical Center, USA in which more than 70% of surfaces inside the patients' rooms were SARS-CoV-2 RNA RT-PCR positive. This study assessed the viral viability but viable viruses were not detected [28].

In addition, another study from Greece reported that SARS-CoV-2 RNA was detected by rRT-PCR on a wide range of environmental surfaces, including an air conditioning filter and ventilation duct, in the wards of the COVID-19 isolation hospital but the study did not assess the viral viability [32]. Furthermore, several studies reported detection of SARS-CoV-2 RNA by rRT-PCR on many high-touch surfaces in the patient areas from different countries and cities such as the United Kingdom (London; viral viability was assessed but viable viruses were not detected) [24], Italy (Milan; viral viability was not assessed) [33], and China (two studies from Wuhan; viral viability was not assessed) [34,35].

Regarding the patient's bathroom in the conducted study, most (10 out of 12; 83.3%) swabs taken before routine cleaning/disinfection were SARS-CoV-2 RNA rRT-PCR positive including doorknob, Light switch, sink, faucet handles, and toilet bowl surface. The sink, faucet handles, and toilet bowl surface of the patient's bathroom were among the hospital zones with the highest detected contamination levels. Similarly, a previous Chinese study to assess air and surface contamination showed that toilet, bowl, and sink samples were positive suggesting that environmental contamination by SARS-CoV-2 through fecal shedding and respiratory droplets can be a potential source for SARS-CoV-2 transmission. Among the limitations of this Chinese study, was the unavailability of viral viability testing by culture [36].

In the current study, swabs taken from the X-ray room before routine cleaning/disinfection were negative for SARS-CoV-2 by rRT-PCR except for the four (33.3%) samples taken from the doorknob and light switch that was positive. This may be explained by their frequent use or handling while wearing contaminated gloves. In the same line with the results

of the performed study but with a lower contamination level, a study from New Jersey, USA reported that no environmental surface samples tested positive by SARS-CoV-2 PCR among 128 samples collected from different surfaces in a radiation oncology clinic before their cleaning/disinfection [37].

Generally, HCWs have better infection prevention and control knowledge and practice compared to the general population. Regarding the swabs collected from the medical staff room (including the PPEs) and the anteroom before routine cleaning/disinfection in the performed study, most (75.0%) of them were negative for SARS-CoV-2 RNA by rRT-PCR while only the four (25.0%) swabs that were collected from the computer keyboard were positive. This may be explained by the frequent use of the computer keyboard, using the keyboard while wearing contaminated gloves, or the difficulty in its proper decontamination. Similarly, a Chinese study showed that hand sanitizer dispensers, self-service printers, keyboards/desktops, doorknobs, and gloves were the most contaminated hospital objects at rates of 20.3%, 20.0%, 16.8%, 16.0%, and 15.4%, respectively. The Chinese study suggested that SARS-CoV-2 contamination of the hospital environmental objects could be an important occupational risk for HCWs [18].

In the conducted study, all (100%) of the swabs collected after routine cleaning/disinfection were negative for SARS-CoV-2 RNA by rRT-PCR. The cleaning/disinfection of the high-touch areas was performed twice daily using chlorine-containing disinfectant (5000 parts per million; ppm). The floors were cleaned and disinfected daily using chlorine-containing disinfectant (1000 ppm). The result of the conducted study suggests that the current decontamination measures, which were ensured correctly and consistently in the hospital, are effective. This was not unexpected considering many studies from different countries reporting no detection of SARS-CoV-2 RNA after surfaces sanitization. The study of Ong and his research team documented the results of SARS-CoV-2 PCR testing of the environmental surfaces and PPEs surrounding three COVID-19 patients in the isolation rooms of a Singapore hospital, and they reported that no air samples were positive and surfaces' swabs were positive before but not after decontamination measures [36]. Furthermore, a Chinese study by Lai and his colleagues reported that no surface specimen tested positive by SARS-CoV-2 PCR among 90 samples collected from different surfaces in healthcare settings after sanitization [38].

Generally, detection of the viral RNA in the hospital environment, could emphasize the urgent need to ensure optimal environmental cleaning, and improve the infection prevention and control precautions within the hospitals with adequate HCWs training [18,39]. The WHO reported that SARS-CoV-2, SARS-CoV, and MERS-CoV are extremely sensitive to detergents and disinfectants but SARS-CoV-2 is relatively stable in the environment [30]. Thus, the WHO and CDC immediately recommended recurrent cleaning/disinfection of highly touched surfaces to contain the ongoing COVID-19 pandemic [30,40]. A recent study concluded that the potential for SARS-CoV-2 transmission through contaminated environmental surfaces is very rare provided standard cleaning procedures and precautions are implemented [41].

The limitations of the current study are as follows. First, the viral viability was not tested by viral culture due to its unavailability at our locality. Second, no air samples were collected. Third, the inability to make a clinical correlation with the patients' conditions due to operational limitations during the outbreak.

Conclusions and Recommendations

The results of the conducted research highlight the importance of hospital environmental SARS-CoV-2 RNA surveillance. The hospital environment is a high-risk area that can be contaminated by SARS-CoV-2. The contamination of hospital environmental surfaces can occur through contact, respiratory, and maybe fecal shedding of the virus. Contamination could have resulted from the viral shedding from infected patients and/or the indirect contact by patients, HCWs, and visitors. To limit this fatal virus transmission, wearing surgical masks, and strict adherence to proper hand hygiene with optimal decontamination of hospital environmental surfaces are essential. Decontamination of hospital environmental surfaces by using chlorine-containing disinfectant at concentrations of 1000-5000 ppm is effective against SARS-CoV-2.

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