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Short Communication

Detection of Novel Coronavirus on the Surface of Environmental Materials Contaminated by COVID-19 Patients in the Republic of Korea

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ABSTRACT

This study aimed to determine the presence of SARS-CoV-2 on surfaces frequently touched by COVID-19 patients, and assess the scope of contamination and transmissibility in facilities where the outbreaks occurred. In the course of this epidemiological investigation, a total of 80 environmental specimens were collected from 6 hospitals (68 specimens) and 2 "mass facilities" (6 specimens from a rehabilitation center and 6 specimens from an apartment building complex). Specific reverse transcriptase-polymerase chain reaction targeting of RNA-dependent RNA polymerase, and envelope genes, were used to identify the presence of this novel coronavirus. The 68 specimens from 6 hospitals (A, B, C, D, E, and G), where prior disinfection/cleaning had been performed before environmental sampling, tested negative for SARS-CoV-2. However, 2 out of 12 specimens (16.7%) from 2 "mass facilities" (F and H), where prior disinfection/cleaning had not taken place, were positive for SARS-CoV-2 RNA polymerase, and envelope genes. These results suggest that prompt disinfection and cleaning of potentially contaminated surfaces is an effective infection control measure. By inactivating SARS-CoV-2 with disinfection/cleaning the infectivity and transmission of the virus is blocked. This investigation of environmental sampling may help in the understanding of risk assessment of the COVID-19 outbreak in "mass facilities" and provide guidance in using effective disinfectants on contaminated surfaces.

Introduction

After the first outbreak of COVID-19 in Wuhan, China, it was determined that the main transmission route of this novel coronavirus was through inhalation of respiratory droplets released from an infected person coughing and sneezing, and/or having direct contact with a contaminated surface. A lot of attention has been placed on personal protection measures whilst practicing respiratory hygiene (observing coughing etiquette, wearing a mask, frequent hand washing). However, reports on the transmission of SARS-CoV-2 to humans from contaminated surfaces are rare. Some studies have demonstrated that aerosolized SARS-CoV-2 can remain viable and infectious for hours and even days on contaminated

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surfaces. SARS-CoV-2 is especially persistence on inanimate surfaces like metal, glass, or plastic where the virus has remained viable and infectious after 9 days [1,2]. Nevertheless, SARS-CoV-2 can be inactivated by following surface disinfection procedures e.g. 62-71% ethanol, 0.5% hydrogen peroxide, or 0.1% sodium hypochlorite for 1 minute [2].

The 7th Korean guideline recommends disinfection and cleaning of potentially contaminated rooms or areas occupied by confirmed COVID-19 patients. In the Republic of Korea, the first confirmed COVID-19 case was reported on 20th January 2020, and early stage epidemiological investigations were carried out by prompt response teams from Korea Centers for Disease Control and Prevention (KCDC), where the focus was on suppressing a small number of the sporadic outbreaks in the country [3].

On the 4th February 2020, nosocomial infections were reported at a hospital in Gwangju metropolitan city (A), whilst group-associated outbreaks from "mass facilities" were reporting secondary and tertiary attacks [4]. To cope with these surges in nosocomial infections, a special medical management team in KCDC was rapidly organized to begin epidemiological investigations and anti-epidemic measures to prevent transmission to other hospitals and medical facilities.

On the 18th February, a COVID-19-confirmed patient (later designated as the 31st case) in a hospital (B), and all her potential contacts (inpatients, outpatients, health workers, patient helpers) were reported by a health center in the metropolitan city of Daegu. Two prompt response teams of KCDC were dispatched to the hospital to scrutinize the etiology of the nosocomial infection. After their initial assessment to block the chain of transmission, the confirmed COVID-19 cases and identified contacts were moved to separated wards of a city-run hospital for the required isolation period of 14 days.

As part of the epidemiological procedures, an investigation of the environmental samples was performed to discern a route of transmission of the coronavirus, and infectious risk for dispersal of the pathogen [3]. Environmental contamination and the mode of transmission by other coronaviruses such as the Middle-East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) is uncertain. Despite this, MERS-CoV RNA was detected on environmental surfaces such as medical devices e.g. X-ray devices, bedrails, and in waiting rooms [5]. It was reported that MERS may spread through poorly ventilated environments as determined using sulfur hexafluoride (SF) tracer gas [6] and computational fluid dynamics analysis [7]. More recently, it has been reported that small virus-laden droplets of SARS-CoV may be displaced by airflow and deposited on equipment such as ventilation equipment, and hospital floors [8].

In this study, the aim was to detect SARS-CoV-2 using reverse transcriptase-polymerase chain reaction (RT-PCR) as part of the epidemiological investigation. The focus was on surfaces frequently touched by COVID-19 patients within the facilities where the outbreak occurred in order to evaluate the spread of COVID-19, and the risk of infection.

Materials and Methods

To assess the presence or absence of SARS-CoV-2 within the congregate care facilities, there were a total of 80 environmental samples collected from surfaces that may be contaminated, irrespective of prior disinfection and cleaning activities (Table 1). The testing was performed between the 4th February and the 5th March 2020.

Environmental samples were taken from an area of 700 cm^2 (30 × 25 cm), which focused on frequently touched surfaces in wards and communal facilities of COVID-19 patients in the hospital. Sterile flexible swabs dipped in viral transport medium (Easy Swab, Komed, SungNam, Korea) were used for detecting SARS-CoV-2 RNA. All environmental samples collected were transported to the laboratory without delay and SARS-CoV-2 RNA was extracted from the viral transport medium using a commercial RNA extraction kit (QIAGEN, Hilden, Germany), according to previous methods by Kim et al (2020) [9].

For identifying the presence of SARS-CoV-2, real-time RT-PCR targeting of RNA-dependent RNA polymerase gene and envelope protein gene were used. The primers and probes were as follows: RNA-dependent RNA polymerase gene detection were: 5'- GTGARATGGTCATGTGTGGCGG-3' (Forward), 5'- CARATGTTAAASACACTATTAGCATA-'3 (Reverse) and 5'-CAGGTGGAACCTCATCAGGAGATGC-3' (Probe in 5-FAM/3'- BHQ format) and the primer and probe sequences used for the envelope protein gene detection were: 5'- ACAGGTACGTTAATAGTTAATAGCGT-3' (Forward), 5'- ATATTGCAGCAGTACGCACACA-3' (Reverse) and 5'-ACACTAGCCATCCTTACTGCGCTTCG-3' (Probe in 5-FAM/3'- BHQ format). A total of 5 mL extracted RNA was reverse transcribed, and amplified by PCR under the following cycle conditions: 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute using an ABI 7500 Fast instrument (Thermo Fisher Scientific) [9].

Cycle threshold refers to the number of cycles required for the fluorescent signal to cross the threshold in RT-PCR and an interpretation of cycle threshold value means a positive result for under 35 cycles, re-test for 36-37 cycles, and negative for over 38 cycles as the same criteria for human diagnosis. A lower cycle threshold value could reversely interpret a higher viral load on the surface.

Table 1. Environmental sampling for detecting SARS-CoV-2in congregate settings (as of 18th March 2020).

Hospital/ facilities	Index case number (gender/age/ occupation)	No. of additionally confirmed cases	No. of potential contacts	Environmental sampling date (2020)	No. of specimen sampled	Prior disinfection/ cleaning before sampling
A hospital	#164 (F/42/inpatient's family member)	1	140	4th Feb.	12	Yes
B hospital	#31 (F/61/inpatient)	2	49	18th Feb	32	Yes
C general hospital	#89 (F/24 / nurse)	7	76	22th Feb	5	Yes
D long-term care hospital	#759 (M/57 /inpatient helper)	31	109	25th Feb	5	Yes
E hospital	#187 (F/50/ inpatient family member)	16	132	26th Feb	8	Yes
F rehabilitation center	#1950 (M/43/teacher)	8	80	1st Mar.	6	No
G clinic	#878 (M/ 62/ inpatient)	1	0	5th Mar	6	Yes
H apartment houses	#31 (F/61/inpatient)	45	94	5th Mar	6	No

Results

A total of 80 environmental specimens were collected from 6 hospitals and 2 "mass facilities" (Table 2). These specimens were from frequently-touched surfaces such as telephones, bedrails, chairs, and door handle in wards, and communal facilities where COVID-19 patients stayed. Among them, 68 samples from 6 hospitals (A, B, C, D, E, and G) had negative SARS-CoV-2 RNA results because disinfection and cleaning had been performed by the local health centers before samples were collected. Even the car key of a COVID-19 patient's automobile (#31 case) was determined to be negative for SARS-CoV-2 RNA test. However, among the remaining 12 samples from 2 "mass facilities" (F and H) where prior disinfection and cleaning procedures had not occurred, 2 samples collected from door handles in the COVID-19 patient's room in the rehabilitation center (F), were positive for SARS-CoV-2 RNA for both target genes (16.7%, 2/12).

Discussion

Public health emergencies require a rapid assessment of the current epidemiological situation for prevention and control measures. Since 2015 with the MERS outbreak in South Korea to efficiently tackle the transmission of newly emerging infectious diseases, 10 prompt response teams within the Korea Centers for Disease Control and Prevention have been in operation. The teams in the event of an outbreak are dispatched to the site of the outbreak as soon as possible, to identify the index case and potential contacts through epidemiological investigations, and isolate the individuals concerned. Based on the field situation and resources available in congregate health settings, "a cohort isolation of potential contacts" measure if necessary, is implemented instead of individual-level isolation.

Korea has experienced more group-associated outbreaks in congregate health settings than in sporadic occurrences. Recently, a clustered outbreak occurred in Eunpyeong St. Mary's hospital in Seoul, Republic of Korea, which instigated the prompt closing of the hospital, and isolation of the contacts to separate wards within the hospital (cohort isolation), and this seemed to have played a role in preventing the nosocomial spread of COVID-19 and spread to the local community [10]. However, there was no assessment for environmental contamination before closing the hospital and isolating individuals. Perhaps, additional epidemiological investigation for environmental samples would be helpful in decision-making and risk assessment of contamination and spreading of

Table 2. Environmental samples and RT-PCR results.

Sites		No. of specimen sampled	No. of positive	Cycle threshold value (RdRp / E)
Patient's room	Telephone	1	-	
	Bedrail	15	-	
	Chair	5	-	
	Door handle	10	2	1st: 32.92 / 34.8 2nd: 27.84 / 31.49
	Switch	1	-	
	Closet	8	-	
	Wall	4	-	
	Pillow & blanket	5	-	
	Remote Control	2	-	
	Refrigerator	2	-	
Patient's car	Steering wheel	1	-	
	Door handle	1	-	
	Gear stick	1	-	
	Car key	1	-	1st: Pendency Retest: Negative
	Dashboard	2	-	
Medical device	Aqueous hanger	1	-	
	X-ray equipment	1	-	
	Bed in CT room	1	-	
	Medical bed	1	-	
	Dialysis machine	5	-	
	Nursing cart	3	-	
Toilet area	Door handle	4	-	
	Switch	1	-	
	Sink	2	-	
Communal material	Water purifier	1	-	
	Computer	1	-	Negative

RT-PCR = reverse transcriptase-polymerase chain reaction; RdRp = RNA-dependent RNA polymerase gene; E = envelope protein gene.

the pathogens in the related areas.

Human coronaviruses SARS-CoV-2 can remain infectious on inanimate surfaces at room temperature for up to 9 days [2]. Significant environmental contamination with SARS-CoV-2 may be a potential medium of transmission and supports an environmental and hand hygiene [11]. Although the effectiveness and application of environmental epidemiological investigations in outbreaks is limited, it is necessary for risk assessment of additional contamination and spreading of the pathogens.

Data on the transmissibility of SARS-CoV-2 from contaminated surfaces to hands has not previously been reported in South Korea. In the present investigation, the identification of SARS-CoV-2 RNA in 2 environmental samples collected before disinfection and cleaning indicates that the transmissibility of this novel coronavirus is worth exploring on fomites to determine the risks of COVID-19 infection.

Our findings have several limitations. Firstly, viral culture examinations were not performed to identify the viability of the coronavirus on surfaces, which would be helpful in estimating the possibility of additional outbreaks, and understanding the mode of transmission. Secondly, as most of the environmental samples in hospitals were collected after disinfection and cleaning SARS-CoV-2 was not detected.

Our result suggests that prompt disinfection and cleaning of potentially contaminated surfaces is an effective infection control measure by reducing the infectivity of coronaviruses and blocking further opportunity of transmission in the congregate healthcare setting.

This summary could help in the understanding of risk assessment of the COVID-19 outbreak in "mass facilities," and guide a disinfection measures in materials that may be contaminated.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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