Environmental investigation of SARS-CoV-2 in a karaoke bar: a survey for a cluster of COVID-19 in Hokkaido, Japan, 2020

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The coronavirus disease 2019 (COVID-19) caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has drastically spread around the world after the initial appearance in Wuhan, China in December, 2019 (1). In Japan, since the first case was detected in January, 2020, the number of the cases has increased. As of November, 2020, the count has reached 100,000 (2). In June, 2020, in a city of Hokkaido, northern islands of Japan, had reported novel three clusters of COVID-19 among elderly people in karaoke bars, which provide the daytime karaoke singing. In one of the bars, the owner and total seven customers (Age > 60 years old) were observed to be infected by SARS-CoV-2. The onset of illness of one of the customers was a day of June (The index day: Day 1). Following, two of the customers developed illness on Day 5 and one of the customers was on Day 10. The rest three others were asymptomatic. Although the owner developed illness on Day 2, the bar had kept open until Day 6.

On Day 7, the bar had been closed to prevent further transmission of the virus. However, the bar was left unsterilized and untouched. On Day 16, we carried out the environmental sampling in the bar to assess the viral contamination after having the consent of a relative of the owner. Until the day of sampling (from Day 7 to Day 16), the temperature of the city ranged between 14.1°C and 23.9°C, and the humidity ranged between 66% and 99% (3). The sampling started at 10:15 and lasted for one hour. During the sampling, the temperature of the room changed from 21°C to 23°C, and the humidity changed from 61% to 72%.

The following is an overview of the bar. Total floor space of the bar was 32.4m^2 (4.5 meters in width × 7.2 meters in depth). The entrance was located at the center of the frontage facing an alley in the restaurant section. No window was observed and the room ventilation had been turned off. In the bar, a few meters of straight counter was placed on

the right side, and the service entrance to the kitchen was at the back end of the counter. The toilet was located at the left of the service entrance. On the left side in the bar, total 5 seating areas with tables and chairs or sofas were observed on the floor. Sofas were mainly placed along the wall.

Environmental sampling was performed at 50 locations in the bar (Table 1). Each surface was carefully wiped in three directions (vertically, horizontally, and diagonally, < 100 cm^2 each) by a sterile cotton swab moistened with virus transport medium (VTM). Each sample was immersed in 1 ml of VTM in aliquoted tubes to prevent drying, and was sealed and kept in cold storage until laboratory testing. Total RNA was extracted from 140 µl of VTM using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). The detection of SARS-CoV-2 RNA was performed by using SARS-CoV-2 Direct RT-qPCR KIT (Takara Bio Inc., Kusatsu, Japan) and LightCycler 480 II System (Roche, Basel, Switzerland) or Thermal Cycler Dice Real Time System III (TaKaRa Bio Inc.).

The RNAs of SARS-CoV-2 were detected in 18 (36%) of 50 environmental samples (Table 1). The cycle quantification (Cq) values ranged from 34.41 to 41.68. The lowest value was detected from the lever of coffee server located on the bar counter. In contrast, total 5 samples indicated over 40 Cq values that was categorized as under the detection limit of the kit (< 5 copies). If the bar room is divided the entrance, the hall and the restroom into the customer's area, and the bar counter and the kitchen into the owner's area, the positive rate of the latter (46.2%, 12/26) was 1.85 times higher than that of the former (25%, 6/24), suggested viral contamination was more widespread in the owner's area. SARS-CoV-2 can be detected in various human fluids, for instance, sputum, nasal swabs, feces, tears and conjunctival secretions (4,5). In this survey, the SARS-CoV-2 RNA was detected on the surfaces of tabletop, separate plates, pillow, door knob, toilet

floor, instruments for singing, tong, handle of refrigerator, face shield, vinyl curtain, cigarette lighter, water faucet and levers of beverage servers. These results indicated the fluids or feces of the patients had contaminated on them. Especially, the positive results for the handle of refrigerator and the levers of beverage servers strongly suggested the owner had spread the virus.

In the hall, the separate plate on a table and the pillow on a sofa were positive for viral RNA. These results suggested the customers suffered from COVID-19 might also contaminated them. In addition, the positive result on the outside of the face shield indicated the possibility that the individuals who communicated closely with the owner had excreted the virus. Protective equipment such as separate plates, vinyl curtains, and face shields would contribute to reduce the aerosol and the droplets dispersion and the transmission of the virus. However, once it is contaminated after equipping, these items also would be the source of further transmission of the virus. Therefore, as well as where the hand touches with high frequency, the place on where the aerosol and the droplets generated by breathing and coughing are likely to be attached should be also sterilized to interrupt the chain of transmission (6,7).

The infectivity of human coronavirus is considered to remain on inanimate surfaces at least for up to 9 days (7). In this survey, we detected the genome of SARS-CoV-2 from environmental samples in the bar where the patients had joined the daytime singing even until 10 days before. Although virus infectivity was not determined in these samples, our results provided useful information to design the strategy of sterilization and to support the protective behaviors in the commercial space. In the future, more precise investigation of the infectivity of viruses attached on various kinds of objects would be needed to better understand the effective protective methods.

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Conflict of interest None to declare.

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727-33.
2. Ministry of Health, Labour and Welfare. About Coronavirus Disease 2019 (COVID-19). Available at < https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/newpage_00032.html>. Accessed January 8, 2021.
3. Japan Meteorological Agency. Historical weather Data. Available at <https://www.data.jma.go.jp/obd/stats/etrn/view/daily_s1.php?prec_no=16&block_no=47411&year=2020&month=07&day=&view=p1>. Accessed January 8, 2021. Japanese.
4. Xia J, Tong J, Liu M, et al. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol. 2020;92:589-94.
5. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323:1843-4.
6. Wang J, Du G. COVID-19 may transmit through aerosol. Ir J Med Sci. 2020;Mar 24:1-2.
7. Kampf G, Todt D, Pfaender S, et al. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Host Infect. 2020;104:246-

51.

Table 1. Locations and items of environmental sampling and the results of RT-qPCR for SARS-CoV-2 RNA

No.	Locations and items	Cq value	No.	Locations and items	Cq value
	Entrance			Around or on the bar counter	
1	Door knob	(-)*	25	Karaoke remote controller	36.88
	Hall		26	Control panel of the karaoke system	36.76
2	Tabletop	39.72	27	Handle and head of microphone-1	37.59
3	Separate plate on table-1 (near side)	(-)	28	Handle of microphone-2	(-)
4	Separate plate on table-1 (opposite side)	(-)	29	Head of microphone-2	40.98
5	Separate plate on table-2 (near side)	(-)	30	Handle of microphone-3	(-)
6	Separate plate on table-2 (opposite side)	(-)	31	Head of microphone-3	(-)
7	Separate plate on table-3 (near side)	(-)	32	Handle of microphone-4	(-)
8	Separate plate on table-3 (opposite side)	(-)	33	Head of microphone-4	(-)
9	Separate plate on table-4 (near side)	39.53	34	TV remote controller	(-)
10	Separate plate on table-4 (opposite side)	39.08	35	Face shield (outside)	41.25
11	Pillow on the sofa	37.58	36	Face shield (inside)	(-)
12	Back of the sofa	(-)	37	Vinyl curtain suspended over the bar counter (kitchen side)	40.76
13	Cigarette lighter on the guest table	(-)	38	Vinyl curtain suspended over the bar counter (guest side)	(-)
14	TV remote controller	(-)	39	Handle of refrigerator	40.37
15	Ventilation duct at the ceiling	(-)	40	Handle and spout of a coffee cup	(-)
	Restroom		41	Handle and spout of a glass	(-)
16	Door knob (outside)	(-)	42	Handle of water pitcher	(-)
17	Door knob (inside)	39.42	43	Tong for ice pail	39.90
18	Faucet	(-)	44	Hand towel after using	(-)
19	Electric switch of the toilet	(-)	45	Cordless telephone	(-)
20	Door knob of the toilet (outside)	(-)	46	Cigarette lighter	40.00
21	Door knob of the toilet (inside)	(-)		Kitchen	
22	Flush lever of the toilet	(-)	47	Water faucet	41.68
23	Toilet seat	(-)	48	Lever of beer server	36.37
24	Floor around the toilet seat	39.45	49	Lever of coffee server	34.41
			50	TV remote controller	(-)

* No Cq value was detected.