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Short communication

Environmental contamination of SARS-CoV-2 on surfaces, air-conditioner and ventilation systems

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ABSTRACT

Background: COVID-19 can be transmitted directly through respiratory droplets or indirectly through fomites. SARS-CoV-2 has been detected on various environmental surfaces, air samples and sewage in hospital and community settings.

Methods: Environmental samples were collected from a ferryboat during a COVID-19 ongoing outbreak investigation and a nursing home and from three COVID-19 isolation hospital wards and a long-term care facility where asymptomatic COVID-19 cases were isolated. Samples were tested by real-time reverse transcriptase–polymerase chain reaction.

Results: SARS-CoV-2 was detected on swab samples taken from surfaces of food preparation and service areas, hospital isolation wards, an air exhaust duct screen, air-conditioning filter, sewage treatment unit and air sample during investigations conducted in response to COVID-19 outbreaks on a ferryboat, nursing home, isolation facility and COVID-19 hospital wards.

Discussion: Food preparation areas and utensils can be contaminated during COVID-19 outbreaks. Respiratory droplets/nuclei from infected persons can be displaced by the air flow and deposited on surfaces. It can be assumed that in the same manner, air flow could transfer and deposit infected respiratory droplets/nuclei from infected persons to the mucous membranes of persons standing against the air flow direction.

1. Introduction

Currently, it is accepted that COVID-19 can be transmitted directly through respiratory droplets or indirectly through fomites (Ong, 2020; World Health Organization, 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected on various environmental surfaces, air samples and sewage in hospital and community settings (Moriarty et al., 2020; Ong, 2020; Wang et al., 2020; Ye et al., 2020).

We collected environmental samples from a ferryboat during a COVID-19 ongoing outbreak investigation with an attack rate of 31.3% (119/380 travelers), a nursing home (attack rate 9.8%, 12/122), three COVID-19 isolation hospital wards and hallways and a long-term care facility where 30 asymptomatic COVID-19 cases were isolated.

2. Methods

The World Health Organization guidelines were considered for sampling (World Health Organization, 2020).

Air samples were collected with a portable air sampler (Sartorius Airport MD8) with air flow set to 50 L per minute and 10 min sampling time. Gelatin membrane filters of 80 mm diameter (Sartorius 17528-80-ACD) were used. After sampling, filters were removed with sterilized forcep and placed in a 50 ml conical tube filled with ¼ strength Ringer's solution.

Surface samples were collected by wearing sterile gloves as follows: swab was removed from the package, wet to the viral transport medium and then an area of 5 cm by 5 cm was swabbed by applying pressure with the swab on the surface and rotating the swab stick, then the swab was

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Table 1
Environmental samples and positive real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) laboratory test results conducted in March and April 2020 in Greece.

Facility	Type of sample	Positive RT-PCR	
		Sampling site (cycle threshold value)	Number of samples
International ferry boat[a]	Surface swab	Ship hospital and cabins air exhaust duct surface and screen to open deck (34)	1
		Hand contact part of flour scoop in use, in galley (26)	1
		Doorknob in toilets entrance near dining room (37)	1
		Passengers' bar counter (35)	1
		Light switch in the ship hospital (34)	1
	Sewage	Sewage holding tank (27)	1
Nursing home[a]	Surface swab	Patient bed side rail (31, 32)	2
		Filter of wall mounted split air conditioner (34)	1
		Outside doorknob of patient room (32)	1
Hospital COVID-19 negative pressure isolation ward and hallways	Surface swab	Bed side surface patient 1 (32)	1
		Washbasin patient 1 (32)	1
		Toilet bowl button patient 1 (36)	1
	Air	2.5 m away from patient 1 with mask off - 0.8 height (36)	1

[a] Samples were collected once a COVID-19 outbreak was identified and before application of cleaning and disinfection measures.

added to the vial that was placed in a self-sealing bag. The self-sealing bag was cleaned with 70% ethanol solution before placed to the transport container. Control samples were collected in the same way as the environmental samples from the potentially contaminated area, including opening the package and removing the swab from the tube, but without sampling any surfaces.

All samples were tested by real-time reverse transcriptase–polymerase chain reaction (RT-PCR). Genetic material extraction was performed with the iPrep™ Purification Instrument (Invitrogen) using the iPrep™ PureLink® Virus Kit (Invitrogen). For the direct qualitative detection of SARS-CoV-2 RNA, multiplex specific real-time RT-PCR was performed with the RIDA®GENE SARS-CoV-2 RUO kit (R-Biopharm, Germany) in ABI STEP ONE real time system. For each sample a 10^{-1} dilution was also analyzed and both samples and their dilutions were analyzed in duplicate.

3. Results and discussion

Table 1 presents the positive laboratory test results and Table 2 the negative laboratory results. SARS-CoV-2 RNA was detected on the air exhaust duct surface and screen of the ship hospital and cabins exhaust to open deck. Respiratory droplets/nuclei from infected persons were displaced and deposited on the air duct and screen of the ship air exhaust that was located in the open deck, three decks above the ship hospital examination area. Air from cabins and toilets of symptomatic and asymptomatic patients were directed towards the same air exhaust duct. SARS-CoV-2 RNA was detected on the filter of the air-conditioner device in the nursing home patient room. Both results demonstrate that respiratory droplets/nuclei from infected persons can be displaced by the air flow and deposited on surfaces (Lu et al., 2020; Ong, 2020). It can be assumed that in the same manner, air flow could transfer and deposit infected respiratory droplets/nuclei from infected persons to the mucous

Table 2
Environmental samples and negative real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) laboratory test results conducted in March and April 2020 in Greece.

Facility	Type of sample	Negative RT-PCR	Number of samples
		Sampling site	
International ferry boat[a]	Surface swab	Grilles at toilet air outlet in cabin of an asymptomatic COVID-19 crew	1
		Corridor handrail	1
		Clean dish stored inside cupboard	1
		Vegetable refrigerator door-handle	1
Nursing home[a]	Surface swab	Air-conditioner grill	2
		Filter of wall mounted split unit air conditioner indoors	1
		Floor outside patient room	2
		Patient room outside doorknob	2
		Patient room inside doorknob	1
		Toilet outside doorknob	2
		Toilet inside doorknob	2
		Toilet bowl	2
		Patient food table	2
Isolation facility for asymptomatic COVID-19 cases[b]	Surface swab	Living room table	1
		Floor at exit	1
		Exit door's glass window 1,8 m height	1
		Wall against nurses' bench 1,8 m height	1
		Nurses bench	1
		Floor opposite living Room 3.5 m	1
		Wall against living room 3.5 m distance, height 1,8 m)	1
		Wall living room 1.80 height	1
		TV, upper frame	1
		TV remote control buttons	1
	Air	Living room at 0,5 m height	1
		3.5 m distance from living room, height 0,8 m	1
		Nurses' bench 1,2 m height	1
		Exit at 0,5 m height	1
Hospital COVID-19 negative pressure isolation ward and hallways	Surface swab	Food table patient 1[c],2[c],3[c]	3
		Central air outlet from negative pressure rooms in roof of hospital	1
		Toilet bowl button at hallway	1
		Toiled door knob at hallway	1
		Light switch in hallway	1
		Keyboard in hallway	1
		Room for equipment storage in hallway	1
		Bench at hallway	1
		Doorknob in hallway	1
		Anteroom bench in hallway	1
		Bed side surface patient 2	1
		Washbasin patient 2	1
		Toilet bowl button patient 1,2	2
		Light switch patient 3	1
		Air outlet 2 m height patient 3	1
		Air outlet 0.4 m height patient 3	1
		Toilet door knob patient 3	1
		Wall 2 m from patient 3	1
		Wall 3 m from patient 3	1
	Air	1-m height and 2.5 m away from patients 1,2 with mask on	2

(continued on next page)

Table 2 (*continued*)

Facility	Type of sample	Negative RT-PCR Sampling site	Number of samples
		1-m height and 2.5 m away from patients 2,3,3 samples with mask off	3
		Central air outlet from negative pressure rooms (roof of hospital) 1-m height	1
		Bench in hallway	1

[a] Samples were collected once a COVID-19 outbreak was identified and before application of cleaning and disinfection measures.

[b] Samples were collected after cleaning and disinfection application.

[c] Patient 1: symptomatic and 10th day of hospitalization. Patient 2: symptomatic and unknown days of hospitalization. Patient 3: asymptomatic and 13th day of hospitalization.

membranes of persons standing against the air flow direction (Lu et al., 2020).

The virus RNA was detected in one out of the 12 air samples collected. The positive air sample was collected at a height of approximately 0.8 m and 2.5 m away from a symptomatic patient not wearing a face mask. Symptomatic patients -if this is tolerated-could be advised to wear a face mask when a person is entering the isolation area. Equipment used for air sampling does not simulate human breathing patterns and therefore cannot ascertain the potential of airborne disease transmission.

SARS-CoV-2 RNA was detected on a food utensil during food preparation and on the bar counter where service of crew and passengers was taking place. It is possible that contamination of these surfaces occurred through the contaminated hands of food handlers either by respiratory excretions and/or faecal matter. The role of asymptomatic food handlers and the potential for transmission has not been studied yet. Food safety rules should be strictly applied including exclusion of symptomatic food handlers from working, strict personal hygiene, frequent cleaning and disinfection of food preparation areas (World Health Organization, 2020). The use of face masks by food handlers handling ready-to-eat food (especially cold food) may be considered as a precautionary measure during the pandemic and until further evidence is available. Currently there is no evidence of such transmission events.

SARS-CoV-2 RNA was detected in the sewage holding tank of the ferryboat. Occupational exposure, through direct contact with sewage or through inhalation of sewage aerosolized particles could occur and therefore personal protective equipment should be worn by ship crew and workers at the port waste reception facilities.

Our study is limited, by the testing of a limited number of samples. It was not possible to implement a consistent organized monitoring protocol to test hypotheses for the routes of transmission in the specific outbreaks context. Further studies could test samples by culture and even sequencing of clinical and environmental strains could be conducted, both for the phylogenetic comparison of the strains and in order to find potential virus variants (World Health Organization, 2020).

4. Conclusions

SARS-CoV-2 RNA was detected on various surfaces in community settings where outbreaks have occurred and in the immediate environment of patients in hospital wards (Table 1). We were not able to quantify or check virus viability, however, other studies have demonstrated that SARS-CoV-2 can persist on various types of surfaces (Kampf et al., 2020). Hand hygiene and environmental cleaning and disinfection can play a key role in interrupting the chain of infection. Currently, there are only a few studies testing virus resistance to chemical and physical cleaning and disinfection methods. Perhaps additional studies are needed to ensure evidence-based cleaning and disinfection protocols. Further studies can investigate the role of environmental contamination in the disease transmission via analytical epidemiological studies and the extent that indirect transmission can have.

Authors contributions

CH and VM conceived of the study, VM, MKo, GR and SS collected samples, MKy, EP and AV conducted laboratory analysis, ST and CH coordinated outbreak investigations, MKo and LK participated in the air sampling design, all authors reviewed and approved the manuscript.

Declaration of competing interest

Authors declared there are no conflicting interests.

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