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Environmental detection of SARS-CoV-2 in hospital rooms in different wards of a university hospital

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SUMMARY

Background: Transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can occur through direct, indirect, or close contact with infected people. However, the extent of environmental contamination is unknown. The nature of the relation between patients' symptoms and SARS-CoV-2 environmental shedding remains unclear. The aim of this study was to assess the relationship between patient coronavirus disease 2019 (COVID-19) status and environmental contamination.

Methods: Between May and November 2020, environmental swabs were taken before and after room disinfection at day 7 after symptom onset in a cohort of patients clinically or biologically diagnosed with COVID-19. Twelve surfaces per room were collected in 13 rooms. Sample analysis was performed by reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 detection [SARS-CoV-2 R-Gene (biomérieux, Marcy l'Etoile, France)]. Clinical data (day of illness, symptoms, RT-PCR results) was collected from the clinical software.

Results: Five medical units were included in the study. Of 156 samples collected in 13 rooms, five rooms (38.5%) presented 11 SARS-CoV-2-positive samples. These positive samples were detected on eight different surfaces. There was no association between detection of SARS-CoV-2 and patient age (P=1) or patient symptoms (P=0.3).

Conclusion: Viral shedding during COVID-19 appears to be unrelated to the presence of symptoms, patient age, and low-value cycle threshold of patient's test. This study supports the evidence for the environmental shedding of SARS-CoV-2 until at least 7 days after symptom onset. It emphasizes the need for strict compliance with contact precautions, hand hygiene, the correct use of personal protective equipment and room disinfection for the routine care of patients with COVID-19.

Introduction

Since late 2019, the emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has led to a severe respiratory infection named coronavirus disease

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2019 (COVID-19) that spread rapidly into a global outbreak and pandemic. The underlying mechanisms of viral transmission in healthcare environments remain unclear. Transmission of SARS-CoV-2 can occur through direct, indirect, or close contact (within 1 m) with infected people [1]. However, direct or close human-to-human contact with infected secretions (e.g. saliva, respiratory droplets) are the main transmission routes [1]. Therefore, droplet and contact isolation precautions are the key recommended precautions for COVID-19 patient care [1]. Other routes of transmission are suspected, such as ocular route [2], faecal oral route [3], airborne, or fomite transmission [4–6]. However, for fomite transmission, the extent of environmental contamination remains unclear. Several studies have described the persistence of SARS-CoV-2 and its viability on surfaces [4–10]. Infectious SARS-CoV-2 has been found on different environmental contaminated surfaces for periods ranging from an hour to days [1]. Numerous studies have described the analysis of surfaces and hospital rooms for the detection of SARS-CoV-2 virus [11]. Yet most failed to address the nature of the relation between patient COVID-19 status and the SARS-CoV-2 environmental shedding [11]. This information is critical for understanding SARS-CoV-2 transmission and developing effective infection control procedures.

The aim of this study was to assess the relationship between patient COVID-19 status and environmental contamination.

Methods

This retrospective, monocentric study was approved by the local institutional review board (IRB number: 22.03.05). From May to November 2020, environmental samplings were performed in different units at Nîmes University Hospital (France) in rooms according to two inclusion criteria: (1) The presence of a patient with clinically or biologically diagnosed COVID-19. The COVID-19 diagnosis occurred between symptom onset (Day 1) and environmental sampling (Day 7). The clinical diagnosis of COVID-19 was based on French Health Society guidelines [12,13] and established by medical doctors. The diagnosis was orientated by the presence of at least one of the following symptoms (fever, persistent cough, fatigue, rhinitis, dyspnoea, headache, nausea, disorientation, oxygen dependence, myalgia, shortness of breath, diarrhoea, abdominal pain, chest pain, sore throat, anosmia or ageusia) and confirmed with chest scan results. The biological diagnosis of COVID-19 was established by reverse transcription polymerase chain reaction (RT-PCR) performed on nasopharyngeal swabs. (2) The patient in the sampled room was at Day 7 after COVID-19 symptom onset (Day 1).

The methodology timeline from symptoms onset (Day 1) to environmental sampling (Day 7) is represented in Figure 1.

Clinical information was collected from the hospital clinical data software: date of onset of symptoms, clinical presentation, patient clinical status at the sampling day, hospitalization date and, when available, patient's RT-PCR date and cycle threshold (Ct) values. The delay between diagnosis and environmental sampling was estimated in days.

Environmental sampling was performed before room disinfection. Each sampled room was occupied by patient at least 24 h prior to disinfection. Following the local health institute recommendations during the pandemic, room disinfection was performed each day by detergent disinfectant, with virucide activity (European Norm 14 476+A2) according to the French infection control society guidelines [14]. To confirm the efficiency of the local disinfectant, a set of rooms were sampled after room disinfection.

Twelve surfaces were sampled in each room in the presence of patients. The close surfaces sampled were: nurse call controller, bedside rail or bed lifting bar, telephone or television remote, overbed table; and the remote surfaces sampled were: bathroom doorknob, bathroom sink, toilet seat, doorknob, air vent, bathroom sink tap and toilet button. These surfaces are among the most touched surfaces by both patients and health workers. Sampling was performed by dry sterile swabs (GongDong, bioMérieux, Marcy l'Etoile, France) covering the whole surface of the different selected points of sampling. After sampling, swabs were dipped in viral transport medium (MicroTest M4RT, Thermo Scientific, Waltham, MA, USA) and vigorously agitated. The medium was immediately transported to the Department of Virology within a 30-min timelapse and stored at 4 °C.

SARS-CoV-2 was quantified from RNA extracted from each clinical and environmental sample using an automated nucleic acid extraction system (Chemagic, PerkinElmer, Waltham, MA, USA). Samples were analysed using RT-PCR (ARGENE SARS-CoV-2 R-Gene, bioMérieux) with a maximum Ct of 40 targeting three viral genes: the N gene of the nucleocapsid, the RNA dependent polymerase gene (RDRP) and the E gene, which is common to all the SARS viruses. Testing and reading were carried out following manufacturers' instructions. At the time of the study, samples were considered negative after 40 cycles of PCR without any

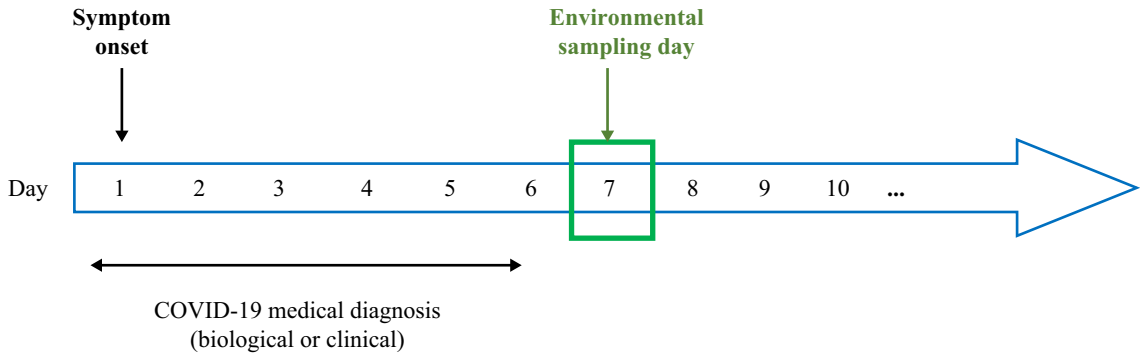

Figure 1. Timeline of sampling environmental in room with patients clinically or biologically diagnosed with coronavirus disease 2019 (COVID-19).

Table I
Sampled rooms, units, and patient's information [symptoms, age and cycle threshold (Ct) values]

Room	Units	Patient information			Positive surface samples			Delay between diagnosis and environmental sampling (days)	Ct (*N*) values of positive surface samples
		COVID-19 symptoms	Age (years)	Ct (*N*) values of patient's test	Close environment	Remote environment	Total		
1	IDU	Asymptomatic	97	25.39	2/5	2/7	4/12	7	38.7; 35.57; 33.20; 35.46
2	NH	Fever, rhinitis, oxygen dependence	84	23.6	0/5	0/7	0/12	7	-
3	NH	Cough, dyspnoea, fever	92	33.88	0/5	0/7	0/12	7	-
4	IDU	Cough, fever, dyspnoea, fatigue	69	37.35	0/5	0/7	0/12	3	-
5	IDU	Cough, fever, dyspnoea, headache	82	29.31	0/5	0/7	0/12	7	-
6	IDU	Cough, fever, dyspnoea, rhinitis	69	34.24	0/5	0/7	0/12	2	-
7	IDU	Cough, rhinitis, headache	83	20.13	1/5	0/7	1/12	2	35
8	ICU	Bilateral pneumonia, oxygen dependence, dyspnoea	45	CD	0/5	0/7	0/12	7	-
9	ICU	Bilateral pneumonia, cough, dyspnoea, fatigue	78	CD	0/5	0/7	0/12	4	-
10	LTCU	Oxygen dependence, dyspnoea, cough, nausea	74	CD	1/5	1/7	2/12	6	37.7; 39.3
11	LTCU	Fever, dyspnoea, oxygen dependence, diarrhoea	76	CD	0/5	0/7	0/12	7	-
12	GU	Fever, cough, disorientation	89	28	1/5	0/7	1/12	3	38.7
13	GU	Fever, cough, disorientation	91	CD	3/5	0/7	3/12	4	31.71; 34.05; 29.75

COVID-19, coronavirus disease 2019; CD, clinical diagnosis; GU, geriatric unit; ICU, intensive care unit; IDU, infectious disease unit; LTCU, long-term care unit; NH, nursing home.

SARS-CoV-2 RNA detection. The Ct value of each gene was recorded. The Ct value of the N gene was used as reference. The delay between swabbing and processing (extraction and RT-PCR) of samples was around 24 h.

Statistical association with environmental contamination was analysed using Fisher's exact test. The distribution of the Ct values of the RT-PCR tests were analysed with a Wilcoxon rank sum test. A *P*-value inferior or equal to 0.05 was considered statistically significant. Statistical analyses were performed using the statistical software R version 4.0.2.

Results

Thirteen rooms were sampled before room disinfection in five medicals units: infectious diseases unit (IDU, $N = 5$), geriatric unit (GU, $N = 2$), intensive care unit (ICU, $N = 2$), long-term care unit (LTCU, $N = 2$) and a nursing home (NH, $N = 2$). Three rooms were sampled in May 2020 during the first wave of the pandemic, four rooms in August 2020 and six other rooms during the second wave in November 2020. At inclusion, five patients were diagnosed on clinical criteria and eight on both clinical and biological criteria. A total of 156 and 52 samples were analysed, respectively, before and after room disinfection. Four rooms were sampled in IDU after room disinfection with a total of 52 analysed samples. Environmental samples were performed an average of 5 days (2–7 days) after nasopharyngeal test (Table I).

Before disinfection, five rooms (38%, 5/13) presented positive samples (Table I). Interestingly, no rooms in the ICU or NH showed environmental SARS-CoV-2 contamination. Positive environmental samples had a Ct range between 29.75 and 39.3 (Table I). Eight samples were positive in the immediate patient environment [nurse call controller ($N = 2$), bedside rail ($N = 2$), bed lifting bar ($N = 1$), telephone ($N = 2$), overbed table ($N = 1$)] and three in patient's remote environment [bathroom doorknob ($N = 1$), bathroom sink tap ($N = 1$), toilet seat ($N = 1$)] (Table II, Figure 2). During room sampling, 12 of 13 patients were symptomatic. The eight patients tested by RT-PCR presented a Ct range between 20.13 and 37.35. However, patients in the contaminated rooms presented low Ct values between 20.13 and 28. Interestingly, the room (Room 1) with the highest number of positive samples (4/12 corresponding to bedside rail, doorknob, bed lifting bar and toilet seat) corresponded to the only asymptomatic patient at the moment of the inclusion (Table I). The mean delay between the patient's nasopharyngeal test and environmental sampling was 4.4 days for positive rooms, and 5.5 days for negative rooms (Table I), without significance (P=0.2897). Environmental contamination showed no significant association with patient age (P=1) or patient symptoms (P=0.3). However, Ct values of nasal RT-PCR were significantly lower than the Ct values of environmental RT-PCR (28.7 vs 38.5, respectively; P=0.0074). Moreover, the patients were significantly older in contaminated rooms than in non-contaminated rooms (mean age = 89 years vs 74; P=0.0046).

After disinfection, no room presented positive samples.

Discussion

This study evaluated the relationship between SARS-CoV-2 positivity of patients and the environmental contamination of their room. We also reported the environmental persistence or

Table II
Positive environmental samples and their locations in patients' rooms

Surfaces	Number of positive samples	Close or remote environment (C or R)
Nurse call controller	2	C
Bedside rail	2	C
Bed lifting bar	1	C
Room telephone	2	C
Overbed table	1	C
Bathroom doorknob	1	R
Bathroom sink	1	R
Toilet seat	1	R
Doorknob	0	R
Air vent	0	R
Bathroom tap	0	R
Toilet button	0	R
Total	11	8 C and 3 R

not of SARS-CoV-2 at day 7 after clinical onset of symptoms in 13 patients diagnosed with COVID-19. Despite daily room disinfection, 7 days after the first COVID-19 symptoms, SARS-CoV-2 was found in five patients' close and remote environment before disinfection. After disinfection, no virus was found. This finding reinforces the need for daily disinfection during the care of a COVID-19 patient and also the prevention of pathogens cross-transmission, as recommended by the French national infection control society [14].

A review found an environmental contamination rate ranging from 0 to 75% in ICU and from 0 to 61% in medical units [11]. Our study showed contamination rates in the lower part of theses ranges, with 7% in medical units and 0% in ICU. Among the five investigated units, three (GU, IDU and LTCU) had room contamination whereas two (ICU and NH) did not. The disinfection protocol and room layout were the same in all units. Moreover, the studied population was similar in terms of age, symptoms and Ct values.

Our study selected patients admitted for COVID-19 at hospital, and surface samples were performed 7 days after symptom onset. Other studies have focused on different populations (healthy asymptomatic workers, patients at a long-term care facility or a nurse facility independently of the time of symptom's onset, passengers, and crewmembers of a ship) and various viral sources (respiratory, rectal, urinary, gastrointestinal tract/stool, blood, breast milk, semen samples, surfaces inside and outside patients' rooms) [11]. These other studies on environmental contamination also assessed different surfaces and used different sampling procedures. In some samples, sampling was made with sterile premoistened swabs [4—8], while we used dry sterile swabs. Moreover, some variations in the laboratory analyses procedures (e.g. extraction kits and targets of RT-PCR) could be observed. For example, only the envelope (E) gene was detected in Zhou *et al.*'s study [6], whereas we detected three genes, increasing the sensitivity of the assay. These differences in methodology hamper direct comparison between studies. However, all these studies corroborated the presence of SARS-CoV-2 on room surfaces, representing a potential source of contamination and cross-transmission.

Interestingly, in our study, positive environmental samples were recovered in one asymptomatic patient room at inclusion, and negative environmental samples were recovered in eight rooms of symptomatic patients. Moreover, no significant association between patient symptoms and environmental contamination was noted. These conclusions have been previously reported [5,6,9]. Thus, environmental contamination appeared unrelated to patient symptomatology, although firm conclusions are not possible as only one asymptomatic patient

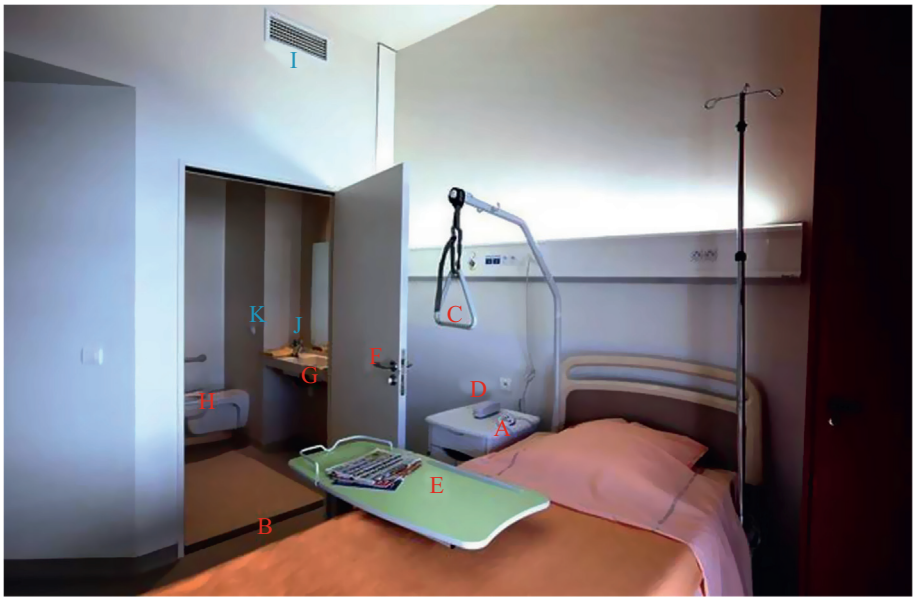

Figure 2. Surfaces sampled in patient's room. A, nurse call controller; B, bedside rail; C, bed lifting bar; D, telephone or television remote; E, overbed table; F, bathroom doorknob; G, bathroom sink; H, toilet seat; I, air exhaust grill; J, bathroom sink tap; K, toilet button. One other point (doorknob) was not pictured. In red, the positive severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) points. In white, the negative SARS-CoV-2 points.

was included. Moreover, for symptomatic patients with environmental contamination, the positive surfaces were mainly located in the patient's close environment (nurse call controller, telephone, bedside rail, bed lifting bar, overbed table), whereas no association was observed between close environment and symptoms. The infectious respiratory secretions produced through coughing or sneezing are the main factor of close contamination [1]. Few studies distinguished close from remote environment when studying environmental contamination. Our results support frequent room disinfection during the hospitalization of a COVID-19 patient, particularly of the immediate close environment or high touch-surfaces, as suggested by the World Health Organization [15]. Notably, despite the significantly greater age of patients in contaminated rooms (mean agen= 89 years vs 74 in non-contaminated rooms; P=0.0046), no significant association between patient age and environmental contamination (P=1) was observed, suggesting that age had no role in environmental contamination, as previously reported [4,10].

All the included patients in this protocol were bedridden and could not use the toilet. In contaminated rooms, SARS-CoV-2 virus was detected in both remote and close patient's environment (Figure 2). Thus, we could suggest that this contamination resulted from: (1) hand contamination either by the patient or the healthcare workers [16] or from the healthcare workers' personal protective equipment (PPE) such as gloves [17]; or (2) respiratory droplets or respiratory aerosols from the patient [4,18]. Another interesting finding is that bedside rail, nurse call controller and room telephone were the most contaminated surfaces. These objects are among the most touched surfaces by both patients and health workers [15]. This reinforces the need for hand hygiene and the correct use of PPE by healthcare workers [15]. This study contributes to the recent debate regarding the duration of the infection control precautions during the hospital care of patients with COVID-19.

Our study has several limitations. Firstly, we did not evaluate the viability of the SARS-CoV-2 collected in the environmental swab samples. Only viable virus can cause an infection. Despite the detection of SARS-CoV-2 virus in the environment, virus viability and infectivity cannot be determined by RT-PCR. Casia *et al*. observed that coronaviruses' infectivity declines in environment with decline rates from 99% at 5 min to 83% at 3 days depending on dried or wet mucine state, while still remaining infectious [19]. In addition, a review has highlighted that given the difficulties in culturing infectious virus from clinical specimens during a pandemic, using viral RNA load as a surrogate remains plausible for generating careful clinical hypotheses [11].

Secondly, our population size is relatively small. Nevertheless, we investigated a representative panel of units and COVID-19-positive patients hospitalized at the time of the study.

In conclusion, our study supports the evidence for the environmental shedding of SARS-CoV-2 until at least 7 days after symptoms onset. Age and patient's RT-PCR Ct values were not significantly associated with room contamination, but the results of this study give a hint that absence of symptoms did not influence environmental contamination. Further studies exploring the link between patient symptoms and environmental contamination would be worthwhile. Our findings emphasize the need for strict compliance with contact precautions, the reinforcement of hand hygiene, the correct use of PPE by health workers and daily disinfection even after 7 days for the routine care of COVID-19 patients, as recommended by health authorities.

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Author contributions

Conceptualization, J.O., A.S. and J.P.L.; methodology, K.B.B., J.O., A.S. and J.P.L.; software, K.B.B.; validation, J.O., A.S. and J.P.L.; formal analysis, K.B.B., J.O., A.B. and R.S.; investigation, K.B.B. and J.O.; writing—original draft preparation, K.B.B., J.O. and J.P.L.; writing — review and editing, A.B., R.S. and A.S.; supervision, A.S. and J.P.L.; project administration, A.S.; funding acquisition, A.S. and J.P.L. All authors read and agreed to the published version of the manuscript.

Conflict of interest statement

None declared.

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References

[1] World Health Organization. Transmission of SARS-CoV-2: implications for infection prevention precautions. Geneva: WHO; 2020. Available at: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions [last accessed January 2021].

[2] Dockery DM, Rowe SG, Murphy MA, Krzystolik MG. The ocular manifestations and transmission of COVID-19: recommendations for prevention. J Emerg Med 2020;59:137—40.

[3] Xiao F, Sun J, Xu Y, Li F, Huang X, Li H, et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. Emerg Infect Dis 2020;26:1920—2.

[4] Chia PY, Coleman KK, Tan YK, Ong SWX, Gum M, Lau SK, et al. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat Commun 2020;11:2800.

[5] Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MSY, et al. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 2020;323:1610.

[6] Zhou J, Otter JA, Price JR, Cimpeanu C, Garcia DM, Kinross J, et al. Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the COVID-19 pandemic in London. Clin Infect Dis 2021;73:e1870—7.

[7] Ren SY, Wang WB, Hao YG, Zhang HR, Wang ZC, Chen YL, et al. Stability and infectivity of coronaviruses in inanimate environments. World J Clin Cases 2020;8:1391—9.

[8] Wu S, Wang Y, Jin X, Tian J, Liu J, Mao Y. Environmental contamination by SARS-CoV-2 in a designated hospital for coronavirus disease 2019. Am J Infect Control 2020;48:910—4.

[9] Wei L, Lin J, Duan X, Huang W, Lu X, Zhou J, et al. Asymptomatic COVID-19 patients can contaminate their surroundings: an environment sampling study. mSphere 2020;5.

[10] Minghui Y, Liang L, Ting H, Shaxi L, Mingxia Z, Yang Y, et al. SARS-CoV-2 detected on environmental fomites for both asymptomatic and symptomatic patients with COVID-19. Am J Respir Crit Care Med 2021;203:374—8.

[11] Kampf G, Brüggemann Y, Kaba HEJ, Steinmann J, Pfaender S, Scheithauer S, et al. Potential sources, modes of transmission and effectiveness of prevention measures against SARS-CoV-2. J Hosp Infect 2020;106:678—97.

[12] Haut Conseil de Santé Publique. Avis relatif aux signes cliniques d'orientation diagnostique du COVID-19. Paris: Haut Conseil de Sante Publique; 2020. p. 18.

[13] Haut Conseil de Santé Publique. Rapport relatif à l'actualisation de la prise en charge des patients atteints de COVID-19 Paris: Haut Conseil de Sante Publique; 2020. p. 291.

[14] Avis SF2H 2019-nCoV — publication de 28 janvier. Available at: https://www.sf2h.net/avis-sf2h-2019-ncov-publication-de-28-janvier-2020; 2020 [last accessed December 2021].

[15] World Health Organization. Cleaning and disinfection of environmental surfaces in the context of COVID-19. Geneva: WHO; 2020. Available at: https://www.who.int/publications-detail-redirect/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-covid-19 [last accessed April 2021].

[16] Hirose R, Ikegaya H, Naito Y, Watanabe N, Yoshida T, Bandou R, et al. Survival of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza virus on human skin: importance of hand hygiene in coronavirus disease 2019 (COVID-19). Clin Infect Dis 2021;73:e4329—35.

[17] Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104:246—51.

[18] Santarpia JL, Herrera VL, Rivera DN, Ratnesar-Shumate S, Reid SP, Denton PW, et al. The infectious nature of patient-generated SARS-CoV-2 aerosol. medRxiv 2020:2020. 07.13. 20041632.

[19] Mucins inhibit coronavirus infection in a glycan-dependent manner. ACS Cent Sci 2022;8:351—60.