European Review for Medical and Pharmacological Sciences 2020; 24: 9202-9207

SARS-CoV-2 RNA contamination on surfaces of a COVID-19 ward in a hospital of Northern Italy: what risk of transmission?

M. D'ACCOLTI[1,2], I. SOFFRITTI[1,2], A. PASSARO[3,4], G. ZULIANI[3,4], P. ANTONIOLI[5], S. MAZZACANE[2], R. MANFREDINI[3,6], E. CASELLI[1,2]

[1]Section of Microbiology, Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy
[2]CIAS Research Centre, University of Ferrara, Ferrara, Italy
[3]COVID-19 Internal Medicine Units, S. Anna University Hospital, Ferrara, Italy
[4]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy
[5]Department of Infection Prevention Control and Risk Management, S. Anna University Hospital, Ferrara, Italy
[6]Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Abstract. – OBJECTIVE: SARS-CoV-2 can reportedly exist on inanimate surfaces for a long duration, but there is limited data available from Italian COVID-19 hospital wards, especially for non-intensive care units hosting patients that do not require mechanical ventilation. Identification of the extent of environmental contamination can help in understanding possible virus transmission routes, limit hospital infections and protect healthcare workers. Thus, we investigated virus contamination on surfaces of the acute COVID-19 ward of an Italian hospital.

MATERIALS AND METHODS: Ward surfaces, including four points inside and six points outside the patients' rooms were sampled by swabs, seven hours after routine sanitation. To minimize the risk of underestimation of virus detection, two different sensitive molecular methods were used comparatively, and specific internal controls were added to enhance the efficiency of all the analysis steps.

RESULTS: SARS-CoV-2 contamination was detected in only three out of all the collected samples, i.e., on two floors and one-bathroom sink, likely reflecting aerosol and saliva contamination, respectively. The overall level of contamination was low, and the floors exhibited a very low level of SARS-CoV-2 presence, evidenced by only one of the two methods used.

CONCLUSIONS: The existence of SARS-CoV-2 on hospital surfaces may be limited, although it was reported to persist for a longer duration on surfaces under controlled laboratory conditions. Thus, effective transmission of SARS-CoV-2 by surfaces/fomites within the hospital ward may be a rare event. However, the results highlight the importance of assessing method sensitivity and including controls when investigating low-level virus contamination so as to avoid the risk of underestimation of virus presence.

Key Words:
SARS-CoV-2, COVID-19, Hospital environmental contamination.

Introduction

Emergency measures aimed to limit the spread of the novel pandemic virus SARS-CoV-2 have been adopted globally due to the current absence of specific vaccines and/or drugs. These measures include social distancing[1], optimization of diagnostic tools[2,3], increased hand hygiene, and increased environmental disinfection[4]. It is known that similar respiratory viruses (including SARS-CoV-1 and MERS) may exist on different inanimate surfaces and that in the hospital environment that may be contaminated by body fluids. This could facilitate the spread of the infection[5-7]. In addition, SARS-CoV-2 was reported to persist on surfaces for days in controlled experimental conditions[8], and its isolation from urine has also been reported[9].

Thus, the contribution of hospital environment to the transmission of COVID-19 infection *via* contact with contaminated surfaces and fomites needs to be elucidated.

However, there is not much information on SARS-CoV-2 contamination in Italian hospitals,

Corresponding Author: Elisabetta Caselli, Ph.D; e-mail: csb@unife.it

especially for acute care units hosting COVID-19 symptomatic patients that do not need mechanical ventilation. At the time of conducting this study, we found that most of the available studies focused on intensive care units, and only one study surveyed Italian healthcare structures[10]. Most of these available studies used single specific molecular assays to detect SARS-CoV-2 RNA, and a few of them attempted virus isolation[11]. However, none of these studies included molecular controls to monitor the efficiency of the analysis steps.

Thus, there is a requirement of data on the level of environmental SARS-CoV-2 contamination in real-life conditions, especially for non-intensive wards where less symptomatic (or totally asymptomatic) patients might contribute consistently to virus spread. This may be due to less awareness of their continuous release of infectious virus, potentially increasing the risk of environmental transmission[12].

Hospitals have mandatorily implemented heavy disinfection measures[13]. Although the only Italian study showed no virus RNA contamination[10], other studies reported contamination by SARS-CoV-2 RNA, even *via* protective personal equipment (PPE) kits of the sanitary staff[14].

Since identifying the exact extent of environmental contamination and associated potential risk of virus transmission is crucial to understanding virus epidemiology, as well as for infection prevention and control in hospitals, we analyzed the presence of SARS-CoV-2 on inanimate surfaces of an acute COVID-19 ward in Northern Italy that hosts symptomatic patients that do not need mechanical ventilation. The analysis was performed seven hours after sanitation to get a representative picture of the contamination degree during the whole day, rather than just after disinfection. Also, two different molecular methods targeting different virus genes were comparatively used for virus detection. A synthetic single-strand RNA (ssRNA) control was also included that allowed the monitoring of the efficiency of each step of the analysis process that might be partially or totally inhibited by the presence of high amounts of disinfectants on surfaces.

Materials and Methods

Sampled Ward

An acute non-intensive COVID-19 ward of the University Hospital of Ferrara (Italy) was surveyed. Cleaning procedures consisted of double daily sanitation (in the early morning around 7:00 am and in the afternoon around 3:00 pm) of the floor using 0.5% sodium hypochlorite and other surfaces using 1% hydrogen peroxide.

The access to the ward was strictly allowed to sanitary staff only, who were required to wear PPE, including liquid-repelling gowns, class-2 filtering face masks with added surgical masks, eye protection (face shield), disposable shoe cover, hair cap, and double gloves. No COVID-19 infections of ward personnel were recorded in the ward from the opening until the time of the investigation. At the time of analysis, the ward hosted 30 symptomatic patients with SARS-CoV-2-positive nasopharyngeal swab at the time of admission, with some of them requiring oxygen therapy. Ward rooms hosting two patients were sampled.

Sampling Procedure

Environmental sampling was performed seven hours after cleaning, by using sterile rayon swabs (Copan, Brescia, Italy) pre-moistened in sterile phosphate-buffered solution (PBS), rubbed on a 10 cm ×10 cm area, as previously reported[15]. Surfaces inside (floor, bedside table, bathroom sink, and bed headboard) and outside (ward corridor, nurse area and door, and warehouse shelves) the patients' rooms were sampled. Sampled points were chosen as representative of microbial contamination of hospital environment, based on previous studies[16-18]. All the collected samples were put in 0.4 ml of 0.1% SDS in PBS, refrigerated, and processed within 2 hours.

Samples Analysis

Samples were vortexed to detach the virus and squeezed by centrifugation through CW spin basket (Promega, Milan, Italy). Total DNA and RNA were extracted from 350 µl of suspension by the DNA/RNA Patho Gene-spin Extraction kit (Generon, Modena, Italy). Prior to extraction, a synthetic Internal Control (IC) ssRNA molecule was added to each sample, in order to allow monitoring the efficiency of subsequent steps.

The presence of SARS-CoV-2 RNA was evaluated by two different specific real-time PCR after retrotranscription (rRT-PCR), targeting the RNA-dependent RNA polymerase (*RdRp*) gene (Generon, Modena, Italy), and the *orf1ab*, *spike (S)*, and *nucleocapsid (N)* genes (ThermoFisher, Milan, Italy), respectively. Positive controls consisted of plasmid DNA containing the targeted virus genes. The sensitivity of both rRT-PCR kits was around 10 copies.

Results

Sample Collection

Overall, 22 samples were collected from the surfaces of the enrolled COVID-19 ward, including areas inside and outside the patients' rooms, as depicted in Figure 1. Each sampled room hosted two SARS-CoV-2-positive patients with mild to severe respiratory symptoms; of them, two patients needing oxygen therapy.

Analysis of SARS-CoV-2 Presence on Surfaces

All samples tested positive for IC control, confirming the appropriate efficiency of the whole analysis process.

By contrast, only 3/22 samples tested positive for SARS-CoV-2 RNA. One sample derived from a bathroom sink was found to be contaminated as per both rRT-PCR methods (Ct values were 29.54 for *N* gene, 30.77 for *S* gene, 31.67 for *orf1ab*, and 32.99 for *RdRp* gene). Two floors tested positive with only one of the two methods used (Ct>35, corresponding to less than 10 copies, for *N* gene, *S* gene, and *orf1ab*; Ct>40 for *RdRp* gene) (Table I).

The positive samples from the sink and one of the floors were derived from a room hosting two self-sufficient patients who did not require oxygen therapy. The other positive floor was from a room hosting two patients, one of whom needed oxygen therapy.

All the sampled points outside the patients' rooms were found free of SARS-CoV-2 RNA.

Discussion

Defining the precise extent of SARS-CoV-2 contamination in the hospital environment is important to assess the potential risk of virus transmission and design appropriate actions for infection prevention/control and protection of healthcare workers.

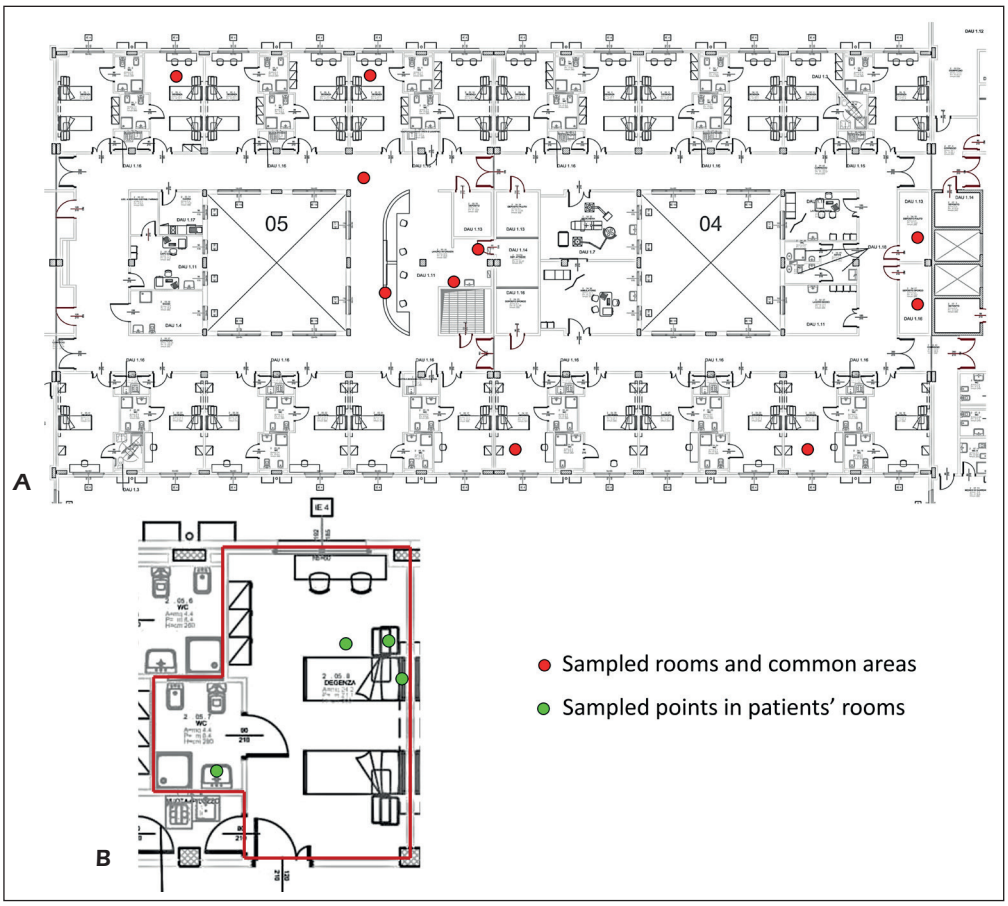

Figure 1. Schematic representation of the surveyed COVID-19 ward. **A**, Ward sampled areas, including four rooms and six common areas (corridor, nurse table, nurse "clean" area, nurse door, shelf of warehouse n.1, shelf of warehouse n. 2). **B**, Room sampled points, including floor, bed headboard, toilet sink, and bed table. Each sampled room was allocated to two patients.

Table I. Environmental SARS-CoV-2 RNA contamination.

Area	Surface	Samples (positive/ total)	rRT-PCR (1)			rRT-PCR (2)	
			N gene	S gene	orf1ab	RdRp gene	IC
Room	Sink	1/4	29.54	30.77	31.67	32.99	23.15
	Floor	2/4	>35	>35	>35	>40	22.78
	Bed headboard	0/4	>40	>40	>40	>40	23.28
	Bed table	0/4	>40	>40	>40	>40	22.95
Ward area	Corridor	0/1	>40	>40	>40	>40	22.86
	Nurse area	0/1	>40	>40	>40	>40	23.01
	Warehouse	0/2	>40	>40	>40	>40	22.81
	Door	0/1	>40	>40	>40	>40	23.15

Results are expressed as mean Ct values detected in the positive samples.

Among the few studies reporting data on COVID-19 wards, only one study was performed in Italy in intensive care and infectious disease units and showed the lack of environmental SARS-CoV-2 RNA contamination[10].

However, virus spread might be paradoxically facilitated even from subjects with mild symptoms who can efficiently release infectious virus despite having scarce symptoms[12,19]. In the hospital environment, such patients are often self-sufficient and can freely move within the room and bathroom areas, which could represent a further favoring factor for virus spread.

Thus, our investigation aimed to assess environmental SARS-CoV-2 contamination in such a hospital ward so as to assess the risk of virus transmission by surfaces and fomites in an acute COVID-19 ward of Northern Italy.

Different areas were sampled inside and outside the patients' rooms, including critical points where microbial contamination is more probable[16]. In contrast to the previously published studies, the present study included two different molecular methods and internal controls to avoid the risk of underestimation of virus RNA detection.

Overall, our findings showed a very infrequent presence of SARS-CoV-2 RNA on ward surfaces, including the surfaces that are close to the head of the hospitalized patients within the patients' rooms (headboard, bed table).

These data are in contrast with data showing longer persistence of SARS-CoV-2 virus on inanimate surfaces[8]. However, those data were obtained in the laboratory under stable controlled conditions, whereas our data were collected in real-life conditions, where the existence of virus on surfaces could be hampered by routine sanitation and unstable environmental conditions. This suggests that virus spread *via* contact with surfaces and fomites may be a rare event.

In fact, only 3 samples from the patients' rooms harbored SARS-CoV-2 RNA, two from one room (bathroom sink and floor) and one from a second room (floor). The highest contamination was detected in a room hosting two self-sufficient patients who did not require oxygen therapy, whereas the other positive floor was derived from a room hosting two patients, one of whom required oxygen therapy.

Bathroom sink contamination may indicate recent accidental contamination by the patient's saliva/sputum, possibly during teeth brushing or mouth gargling, and highlights the patients' sputum on surfaces as a potential source of infection. Besides, floor contamination is compatible with contamination due to respiratory droplets and respiratory aerosols, supporting the recent data obtained in Singapore hospitals[20].

No common areas outside the rooms evidenced any traces of virus contamination.

Notably, only the bathroom sink tested positive with both the used molecular assays, whereas the floors resulted positive only by the assay targeting three virus genes. Overall, a low-level contamination was detected, suggesting that the choice of sensitive methods of investigation could be crucial in characterizing potentially poorly contaminated samples as some methods might underestimate the contamination level. This might also apply to the use of such molecular methods for disease diagnosis when defining virus-cleared subjects as not all the molecular methods are equally sensitive. Thus, it is essential to use more specific methods and specifically designed internal controls to identify the risk of underestimation due to non-efficient extraction/amplification.

Conclusions

Limitations of our study include the lack of direct virus isolation to ascertain whether the RNA-positive samples harbored infectious virus, small sample size, and lack of investigation at different time points due to difficulties in performing more tests during the time of emergency.

However, our results suggest that virus spread *via* contact with surfaces or fomites may be rare in real-life conditions and highlight the relevance of sanitation in limiting virus contamination in hospitals, as well as other confined environments.

Acknowledgments

We thank all the sanitary staff of the COVID-19 ward for their active collaboration.

Funding

The research was partially supported by FAR-2019 of the University of Ferrara (PI Elisabetta Caselli).

Conflict of Interests

The authors declare no conflict of interest.

References

1) Cerbara L, Ciancimino G, Crescimbene M, F. La Longa, Parsi MR, Tintori A, Palomba R. A nation-wide survey on emotional and psychological impacts of COVID-19 social distancing. Eur Rev Med Pharmacol Sci 2020; 24: 7155-7163.
2) Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Lee SS. SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnostic and proposed therapeutic options. Eur Rev Med Pharmacol Sci 2020; 24: 4016-4026.
3) WHO. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf (accessed April 3, 2020).
4) WHO. Cleaning and disinfection of environmental surfaces in the context of COVID-19. https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-inthe-context-of-covid-19 (accessed July 10, 2020).
5) Shiu EYC, Leung NHL, Cowling BJ. Controversy around airborne versus droplet transmission of respiratory viruses: implication for infection prevention. Curr Opin Infect Dis 2019; 32: 372-379.
6) Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH, Jr. Survival of influenza viruses on environmental surfaces. J Infect Dis 1982; 146: 47-51.
7) Chowell G, Abdirizak F, Lee S, Lee J, Jung E, Nishiura H, Viboud C. Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. BMC Med 2015; 13: 210-222.
8) van Doremalen N, Bushmaher T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Azaibi T, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020; 382: 1564-1567.
9) Sun J, Zhu A, Li H, Zheng K, Zhuang Z, Chen Z, Shi Y, Zhang Z, Chen S, Liu X, Dai J, Li X, Huang S, Huang X, Luo L, Wen L, Zhuo J, Li Y, Wang Y, Zhang L, Zhang Y, Li F, Feng L, Chen X, Zhong N, Yang Z, Huang J, Zhao J, Li Y. Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. Emerg Microbes Infect 2020; 9: 991-993.
10) Colaneri M, Seminari E, Piralla A, Zuccaro V, Di Filippo A, Baldanti F, Bruno R, Mondelli MU, the COVID19 IRCCS San Matto Pavia Task Force. Lack of SARS-CoV-2 RNA environmental contamination in a tertiary referral hospital for infectious diseases in Northern Italy. J Hosp Infect 2020; 105: 474-476.
11) Wang J, Feng H, Zhang S, Ni Z, Ni L, Chen Y, Zhuo L, Zhong Z, Qu T. SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus disease 2019 outbreak in a Chinese hospital. Int J Infect Dis 2020; 94: 103-106.
12) Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, Shaman J. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 2020; 368: 489-493.
13) 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: 246-251.
14) Ong SWX, Tan YK, Chia PY, Lee TH, Ng OT, Wong MST, Marimuthu K. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 2020; 323:1610-1612.
15) Caselli E, D'Accolti M, Vandini A, Lanzoni L, Camerada MT, Coccagna M, Branchini A, Antonioli P, Balboni PG, Di Luca D, Mazzacane S. Impact of a probiotic-based cleaning intervention on the microbiota ecosystem of the hospital surfaces: focus on the resistome remodulation. PLoS One 2016; 11: e0148857.
16) Caselli E, Brusaferro S, Coccagna M, Arnoldo L, Berloco F, Antonioli P, Tarricone R, Pelissero G, Nola S, La Fauci V, Conte A, Tognon L, Villone G, Trua N, Mazzacane S, the SAN-ICA Study Group. Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: A multicentre, prospective, intervention study. PLoS One 2018; 13: e0199616.

17) D'Accolti M, Soffritti I, Mazzacane S, Caselli E. Fighting AMR in the healthcare environment: microbiome-based sanitation approaches and monitoring tools. Int J Mol Sci 2019; 20: 1535-1548.

18) Comar M, D'Accolti M, Cason C, Soffritti I, Campisciano G, Lanzoni L, Bisi M, Volta A, Mazzacane S, Caselli E. Introduction of NGS in environmental surveillance for healthcare-associated infection control. Microorganisms 2019; 7: 708-729.

19) Lavezzo E, Franchin E, Ciavarella C, Cuomo-Dannenburg G, Barzon L, Del Vecchio C, Rossi L, Manganelli R, Loregian A, Navarin N, Abate D, Sciro M, Merigliano S, De Canale E, Vanuzzo MC, Besutti V, Saluzzo F, Onelia F, Pacenti M, Parisi S, Carretta G, Donato D, Flor L, Cocchio S, Masi G, Sperduti A, Cattarino L, Salvador R, Nicoletti M, Caldart F, Castelli G, Nieddu E, Labella B, Fava L, Drigo M, Gaythorpe KAM, Imperial College COVID-19 Response Team, Brazzale AR, Toppo S, Trevisan M, Baldo V, Donnelly CA, Ferguson NM, Dorigatti I, Crisanti A. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. Nature 2020: 584: 425-429.

20) Chia PY, Coleman KK, Tan YK, Ong SWX, Gum M, Lau SK, Lim XF, Lim AS, Sutjipto S, Lee PH, Son TT, Young BE, Milton DK, Gray GC, Schuster S, Barkham T, Pratim De P, Vasoo S, Chan M, Peng Ang BS, Tan BH, Leo Y-S, Ng O-T, Wong MSY, Marimuthu K, for the Singapore 2019 Novel Coronavirus Outbreak Research Team. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat Commun 2020; 11: 2800-2807.