Environmental Contamination with SARS-CoV-2 in Hospital COVID Department: Antigen Test, Real-Time RT-PCR and Virus Isolation

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Abstract: Background: With the worldwide outbreak of the COVID-19 pandemic, an important question about virus transmission via contaminated surfaces is arising; therefore, research is needed to prove the persistence of viable viruses on surfaces. The purpose of the study was to determine the level of surface contamination with SARS-CoV-2 in a university clinical center. Methods: A study of environmental viral contamination in the rooms of an acute COVID department was performed. Rapid qualitative antigen tests, real-time RT-PCR, and virus isolation in cell cultures were used for virus detection. Results: None of the taken samples were antigen positive. The SARS-CoV-2 RNA was detected in 10% of samples: one positive sample in an empty room after cleaning and disinfection; nine positive samples in occupied rooms. No viable virus was recovered on cell cultures. Conclusions: In our research, the rapid antigen tests did not prove to be effective for environmental samples, but we were able to detect SARS-CoV-2 RNA in 10% of samples using the RT-PCR method. The highest proportion of PCR-positive samples was from unused items in occupied multi-bed rooms. No viable virus was detected, therefore, infection by surface transmission is unlikely, but it remains prudent to maintain strict hand and environmental hygiene and the use of personal protective equipment.

Keywords: SARS-CoV-2; hospital environment; antigenic tests; real-time RT-PCR; virus isolation

1. Introduction

Equipment and surfaces in hospitals and healthcare facilities represent a possible way for the transmission of microorganisms between patients and healthcare professionals, so cleaning, disinfection or sterilization of reusable surfaces and objects is recommended for the control and prevention of healthcare-associated infections [1–3]. With the worldwide outbreak of the novel coronavirus disease in 2019, caused by the pathogen SARS-CoV-2, an important question about virus transmission via contaminated surfaces arose. Direct exposure to respiratory droplets is the major route of SARS-CoV-2 transmission, but a contaminated health environment could potentially lead to SARS-CoV-2 transmission [4,5], as proposed in the case of other coronaviruses (SARS-CoV, MERS-CoV) [6–9]. Much research related to the risks of infection or transmission of the SARS-CoV-2 virus via contaminated surfaces has been performed recently, where the majority concluded as a low probability or unlikeliness [10–17]. Coronaviruses, including SARS-CoV-2, have been shown to survive for hours or days on environmental surfaces [18,19], however, these and similar experiments were performed under laboratory conditions by testing the survival of infectious viruses for days following inoculation of various surfaces. Such studies have been
shown to have no relevance to real-world environments [20] due to high concentrations of inoculums that are not realistically expected in a real-life setting [11] and also due to optimal laboratory conditions (i.e., temperature and humidity) [21], whereas these are variables in the real world. Alternatively, many studies have been performed in the past two years to determine the persistence of viral RNA on surfaces [8,14,15,22–31].

However, further research is needed to assess the persistence of viable viruses on surfaces [6] since only a few studies use the virus isolation method or even parallel, compare and verify the RT-PCR positive samples with the virus isolation method [14,32–36]. The purpose of the study is to determine the level of surface contamination with SARS-CoV-2 in an acute COVID department at a university clinical centre. Rapid antigen test, real-time RT-PCR and virus isolation methods were used in parallel swabs.

2. Materials and Methods

2.1. Study Design

We performed a prospective observational study of environmental viral contamination in the rooms of the acute COVID Department at Slovenian University Clinical Centre. The selected surfaces in the rooms were screened between October 2021 and February 2022, at three different occasions (time points: 17 November 2021, 9 December 2021, and 13 January 2022). Two kinds of multiple patients’ rooms (up to 5 patients) were screened: (1) empty room at the acute COVID Department after cleaning and disinfection; (2) occupied room at the acute COVID Department. The patients from two rooms share one bathroom. In the occupied rooms, we sampled beds and devices used by patients, as well as beds and devices that were not in use at the time of sampling (but were installed in multi-bed rooms with the patients).

2.2. Occupied Rooms Conditions

The rooms have an entrance hall; no passive ventilation is established. Ventilation takes place through windows; 10 min of ventilation several times a day. Cleaning and disinfection of the patient’s immediate surroundings in rooms where isolation measures are in place is performed by medical staff at least three times a day and, if necessary, when visibly contaminated. For surface disinfection, 70% ethanol was used only on cleaned surfaces. For dirty conditions, Clinell universal wipes with active ingredients of didecyl dimethyl ammonium chloride, benzalkonium chloride, 2-phenoxyethanol and polyhexamethylene biguanide [37] were used. Both disinfectants are ready to use and no further dilution is required. The contact time of 70% ethanol is 1 min, and that of Clinell universal wipes is 2 min (tested in dirty conditions). The Clinell universal wipes are tested according to the European Standard EN 14476:2013 + A2:2019 principle for virucidal activity against enveloped viruses. The cleaning service used the cleaning/disinfectant Taski sprint antibac for cleaning [38] with virucidal effect according to EN 14476, contact time 5 min and active component alkyl (C12-16) dimethylbenzyl ammonium chloride 70 g/kg.

2.3. Sampling

Surface samples were obtained with a pre-soaked swab (using supplied buffer according to the manufacturer’s instructions) on the surface area of approximately 100 cm² and used to perform the rapid qualitative test Rapid Surface Ag 2019-nCoV (Prognosis Biotech, Larissa, Greece). At the same time, the second swab was taken (soaked with sterile saline solution-0.9% NaCl) on the same surface directly next to the sampling site for the antigen test, but this time with a HiViralTM Transport Kit (HiMedia, Mumbai, India) for RNA extraction and virus isolation. Sampling locations included hospital bed (headboard), bed railing, overhead trapeze, bedside table, infusion stand, calling device, faucet, zipper on the partition wall, closet and toilet bowl cover.
2.4. Antigen Tests, RNA Extraction, Real-Time RT-PCR and Virus Isolation

The antigen test Rapid Surface Ag 2019-nCov (Prognosis Biotech, Larissa, Greece) were performed on site. The samples collected with HiViralTM Transport Kit (HiMedia, Mumbai, India) were sent to the laboratory. Swabs were vortexed and 600 µL of HiViral™ transport medium was transferred into barcoded secondary tubes, loaded on the cobas® 6800 system. Cobas® 6800 system is fully automated sample preparation, nucleic acid extraction, followed by real-time RT-PCR and detection. For SARS-CoV-2 detection cobas® SARS-CoV-2 kit was used and tested following the manufacturer’s instructions. The remaining volume of 1400 µL HiViral transport medium was frozen at −80 °C for later virus isolation on cell culture, where only PCR-positive samples were tested. Virus isolation on cell culture was performed in a biosafety level 3 laboratory (BSL3). SARS-CoV-2 positive samples were inoculated on Vero E6 cells, incubated at 37 °C in 5% CO₂ incubator for one week and observed daily for the cytopathic effect (CPE). Two blind passages on cell culture were performed for each sample. The CPE was observed under an inverted microscope (Eclipse Ts2R, Nikon, Tokyo, Japan).

3. Results

A total of 100 individual samples were obtained from 10 different patient rooms (2 empty rooms, 8 occupied rooms). Detailed data on department occupancy and the percentage of positive samples are presented in Table 1.

Table 1. Conditions at the hospital’s acute COVID department at the time of sampling.

| Date of Sampling | Number of Sampled Rooms | Rooms Occupancy | Department Occupancy | Number of Samples | Number of PCR Positive Samples/Percentage |
|------------------|--------------------------|-----------------|----------------------|------------------|------------------------------------------|
| 17 November 2021 | 2                        | 11              | 48                   | 32               | 1/3, 13%                                 |
| 9 December 2021  | 6                        | 27 (sampling in the immediate vicinity of 11 patients) | 54                   | 32               | 3/9, 38%                                 |
| 13 January 2022  | 2                        | 12 (sampling in the immediate vicinity of 8 patients) | 33                   | 36               | 6/16, 66%                                |

None of the taken samples were antigen positive. But we were able to detect SARS-CoV-2 RNA in 10 samples (10%), namely: 1 PCR positive sample (5.56%) in an empty room in the COVID department after cleaning and disinfection; 9 PCR positive samples (10.98%) in occupied rooms at the COVID department. Out of those 9 samples, 6 PCR positive samples (33.33%) were detected on unoccupied beads and devices in multi-bed rooms with the patients and 3 PCR positive samples (4.68%) on the occupied beads and devices. The Ct values in all positive real-time RT-PCR samples were >30 (average 33.7). Although viral RNA was detected in 10/100 environmental samples (10%), the propagation of viable virus from PCR-positive samples inoculated on Vero-E6 cells was unsuccessful. No viable virus was recovered from PCR-positive samples.

Detailed information about environmental sampling results from the acute COVID department of the University Clinical Centre are presented in Table 2.
Table 2. Environmental sampling results from acute COVID Department of University Clinical Centre.

| Sampled Area                        | Sampled Object          | No. of RNA-Positive Samples/Total No. of Samples (%) | Real-Time RT-PCR Ct Value Range of Positive Samples (Average) |
|-------------------------------------|-------------------------|-----------------------------------------------------|-------------------------------------------------------------|
| Empty room at acute COVID department | Empty hospital bed      | 0/4                                                 |                                                             |
|                                     | Bedside table           | 1/4 (25%)                                           | 33.8                                                        |
|                                     | Closet                  | 0/2                                                 |                                                             |
|                                     | Overhead trapeze        | 0/2                                                 |                                                             |
|                                     | Calling device          | 0/2                                                 |                                                             |
|                                     | Infusion stand          | 0/2                                                 |                                                             |
|                                     | Faucet                  | 0/1                                                 |                                                             |
|                                     | Zipper on the partition wall | 0/1                                           |                                                             |
|                                     | **Total**               | **1/18 (5.56%)**                                    |                                                             |
| Occupied room at acute COVID department-unused items | Hospital bed          | 3/6 (50%)                                           | 32.2                                                        |
|                                     | Bedside table           | 1/5 (20%)                                           | 36.8                                                        |
|                                     | Closet                  | 0/3                                                 |                                                             |
|                                     | Calling device          | 0/1                                                 |                                                             |
|                                     | Overhead trapeze        | 2/3 (66.67%)                                        | 35.2                                                        |
|                                     | **Total**               | **6/18 (33.33%)**                                   |                                                             |
| Occupied room at acute COVID department-items in use | Occupied hospital bed  | 1/15 (6.67%)                                        | 34.6                                                        |
|                                     | Bed railing             | 1/6 (16.67%)                                        | 33.8                                                        |
|                                     | Bedside table           | 0/20                                                |                                                             |
|                                     | Calling device          | 0/3                                                 |                                                             |
|                                     | Overhead trapeze        | 1/13                                                | 35.0                                                        |
|                                     | Infusion stand          | 0/2                                                 |                                                             |
|                                     | Faucet                  | 0/2                                                 |                                                             |
|                                     | Toilet cover            | 0/2                                                 |                                                             |
|                                     | Door handle             | 0/1                                                 |                                                             |
|                                     | **Total**               | **3/64 (4.69%)**                                    |                                                             |

4. Discussion

In this study, 100 parallel swabs were used to detect SARS-CoV-2 in environmental samples from an acute COVID department at a university clinical hospital using rapid antigen test, real-time RT-PCR and virus isolation methods. In our research, the tests did not prove to be effective despite the manufacturer’s assurance that SARS-CoV-2 nucleocapsid protein antigens could be detected in swabs from common surfaces, such as metal, plastic, glass, paper, and stainless steel [39]. This could be because of the low viral load, as also stated in the Guidance for Antigen Testing for SARS-CoV-2 [40], and was later confirmed with a high real-time RT-PCR Ct value range of positive samples (>30). Sensitivities of the rapid antigen test were found to be positively correlated to the adapted cycle thresholds (Ct) of real-time RT-PCR in clinical samples [41].

We were able to detect SARS-CoV-2 RNA in 10% of samples, which to some extent, correlates to other similar studies [16,32–35,42,43]. No correlation was observed between the percentage of positive samples and the department occupancy. The highest proportion of PCR-positive samples was detected in swabs from unused items in occupied multi-bed rooms (33.33%), which coincides with the findings of Razzini et al. [42] that the samples positivity rate was higher in contaminated and semi-contaminated areas. Cleaning and
disinfection of the patient’s immediate surroundings was performed at least three times a day, but unused objects in multiple bed rooms were not being disinfected. Knowing that the hospitalized infected persons that have the COVID-19 disease with high viral loads in the respiratory tract can release smaller droplets via coughing or sneezing [44] and that such droplets can travel meters or tens of meters long distances in the air indoors by aerosol transmission [45], it is possible for the virus to be transmitted from patients in a multi-bed room.

PCR-positive samples were tested for the presence of viable viruses using propagation in Vero-E6 cells, where no viable virus was detected. This is consistent with other similar studies [16,34,46], which again can be explained by the relatively high CT values (>30) in tested samples. Viable SARS-CoV-2 virus could be cultured from experimentally contaminated dried surfaces with a Ct value < 30, and a lower Ct value has been shown to correlate with successful virus isolation in cell culture [34,43,47,48].

Although infection by surface transmission is unlikely, it is prudent to maintain a strict hand and environmental hygiene regime and use personal protective equipment [34,42,49,50]. Knowledge of viral contamination of surfaces, whether through symptomatic, asymptomatic, or healed patients, has been highlighted by the World Health Organization as an important factor in developing strategies to control outbreaks of viral infections [51].

Our study has some limitations since we investigated a limited number of rooms only in the acute COVID Department and the sampling was conducted during the peak of the pandemic. Therefore, our results cannot be generalized to other areas in healthcare facilities. We also did not simultaneously test patients who occupied investigated rooms, so we cannot correlate patients’ infectivity with environmental viral contamination. The strength of our study was the use of parallel environmental sampling and SARS-CoV-2 detection using rapid antigen test, real-time RT-PCR and virus isolation method.

5. Conclusions

Conclusively, the main finding of this research supports the results of similar studies, that risk of SARS-CoV-2 transmission through contact with contaminated surfaces is low.

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