SARS-CoV-2 Environmental contamination in hospital rooms is uncommon using viral culture techniques

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Abstract

We assessed environmental contamination of inpatient rooms housing COVID-19 patients in a dedicated COVID-19 unit. Contamination with SARS-CoV-2 was found on 5.5% (19/347) of surfaces via RT-PCR and 0.3% (1/347) of surfaces via cell culture. Environmental contamination is uncommon in hospitals rooms; RNA presence is not a specific indicator of infectious virus.

Keywords: COVID-19, Environmental Contamination, SARS-CoV-2, Pandemic.

Introduction

Over 44 million confirmed cases of coronavirus disease 2019 (COVID-19) have occurred as of October 1st 2021.[1] The primary route of SARS-CoV-2 transmission is exposure to respiratory droplets. However, SARS-CoV-2 can persist on environmental surfaces in the laboratory and healthcare setting.[2,3] Therefore, SARS-CoV-2 contaminated surfaces in the healthcare environment could potentially result in transmission of SARS-CoV-2. Previous studies have mainly assessed healthcare environmental SARS-CoV-2 contamination utilizing RT-PCR as a marker for contamination. However, few have utilized cell culture since this work is required to be completed in one of fourteen biosafety level 3 (BSL3) laboratories in the United States[4] Additionally, even fewer studies have used RT-PCR and cell culture in parallel, so the correlation of these methods has been inadequately assessed. The objective of this study was to assess SARS-CoV-2 hospital room contamination and compare the presence of SARS-CoV-2 RNA to infectious virus.

Methods

We performed a prospective observational study of inpatient rooms housing patients with SARS-CoV-2 infection in a dedicated COVID-19 unit at Duke University Hospital Durham, North Carolina. Patient rooms were screened and enrolled in the study between October 2020 and June 2021.

Inpatient Room Conditions

Eligible patient rooms included rooms housing a COVID-19 positive patient with a first positive SARS-CoV-2 test within 24 hours of enrollment. Admission testing for COVID-19 was completed on all patients at the study hospital. All rooms were single-occupant rooms. A previous study demonstrated that the air inside these patient rooms was exchanged roughly 14 times every hour, which is higher than the American Society of Heating, Refrigerating and Air-Conditioning Engineers–recommended ventilation (i.e., ≥6 air exchanges per hour for recovery rooms or ≥12 air exchanges per hour for airborne infection isolation).[5] Routine disinfection was performed in rooms while COVID-19 patients were occupying them. Terminal disinfection in these rooms included bleach solutions for the floor and surfaces followed by ultraviolet C (UV-C) light treatment.

Environmental Sample Collection

Environmental samples were obtained on study day 1 (within 24 hours of the first positive SARS-CoV-2 test) and again on days 3, 6, 10 and 14. Rooms were excluded if samples were not obtained on study days 1 and 3. Surface samples were obtained with nylon FLOQSwab® (Copan, Murrieta, California) pre-moistened with viral transport media (VTM) (Redoxica, Little Rock, Arkansas) from six locations with a pre-defined surface area of 20x20 cm inside the patient room (each bedrail, sink, medical prep area, room computer, exit door handle)and one outside the patient room (nursing station computer) and stored in 1.5mL of VTM during transport.

RNA Extractions, RT-PCR and Viral Culture

Sample swabs were vortexed for 10 seconds to remove viral particles from the swab. Swabs were discarded and RNA extractions were completed on the vortexed VTM using QIAamp® Viral RNA Mini Kits (Qiagen, Hilden, Germany). RT-PCR was completed on all samples using the US Centers for Disease Control and Prevention’s 2019-nCoV Real-Time RT-PCR (reverse-transcription polymerase chain reaction) assay protocol targeting the viral nucleocapsid (N) gene.[6] SARS-CoV-2 virus culture work was performed in a BSL3 laboratory at the Duke Regional Biocontainment Laboratory. Samples positive for SARS-CoV-2 via RT-PCR were inoculated onto Vero E6 cells in 2 passages by transferring 250µL of supernatant at 7 days post inoculation for a total 14 days of incubation. Cells were monitored for cytopathic effect (CPE) every 48 hours. The cells and supernatant were harvested 14 days post-inoculation and were screened for SARS-CoV-2 by molecular assay. Infectious SARS-CoV-2 was confirmed when CPE was detected in inoculated wells and SARS-CoV-2 was detected in inoculated wells by real-time RT-PCR, at least 2 cycle thresholds (Cts) below the original sample. The SARS-CoV-2 isolate USA-WA1/2020 (BEI Resources, Manassas, Virginia) was used as the positive control.

Analysis

Study data were collected and managed using REDCap electronic data capture hosted at Duke University. The demographic characteristics of the study population were summarized using descriptive statistics. This study was deemed exempt non-human research by the Duke University Institutional Review Board and institutional health, safety, environment protocols for culturing of SARS-CoV-2 virus was strictly adhered to, including BSL-3 requirements.

Results

We enrolled 20 patients between October 2020 and June 2021. Patients in these rooms had a median age of 65 (Interquartile range [IQR], 50-73) and 12 (60%) were female. The median length of hospital stay was 6 days ([IQR], 3-11), the median length of stay in the study room was 5 days ([IQR), 3-12) and 16 (80%) of study rooms were previously occupied by a COVID-19 positive patient. 11 (55%) of patients were on supplemental oxygen, and 15 (75%) were actively symptomatic: 8 (40%) pyrexia, 6 (30%) cough, 8 (40%) shortness of breath, and 5 (25%) diarrhea.

A total of 347 individual samples were obtained from 20 patient rooms and screened for SARS-CoV-2 RNA;140 on day 1, 140 on day 3, 48 on day 6, and 14 on day 10. Overall, 19 (5.5%) samples were positive via RT-PCR;9 from bedrails (9.2%), 4 from sinks (8.0%), 4 from room computers (8.0%), 1 from the medical prep area (2.0%) and 1 from the exit door handle (2.0%). Notably, all nursing station computer samples were negative (Figure 1). Of the 19 positive samples, 6 were from day 1, 10 on day 3, 2 on day 6 and 1 on day 10. All 19 SARS-CoV-2 RNA positive samples were screened for infectious virus via cell culture. Notably, only one (0.3%) sample, obtained on day 3 from the bedrails of a symptomatic patient with diarrhea and a fever, demonstrated CPE and the harvested inoculates were SARS-CoV-2 RT-PCR positive, indicating viral growth.

Discussion

The primary route of SARS-CoV-2 transmission is exposure to respiratory droplets. However, SARS-CoV-2 contaminated surfaces in the healthcare environment could potentially lead to indirect transmission of SARS-CoV-2. In our study, the frequency of environmental contamination of SARS-CoV-2 in rooms housing COVID-19 infected patients was low (19/347.5.4%) via RT-PCR and lower (1/347,0.3%) via cell culture.

In general, our results are similar to previous studies.[7] For example, Colaneri et. al found SARS-CoV-2 RNA in 7.7% of environmental samples. [3,8] Our RT-PCR results differed from some studies such as Zhou et al. (52.3% positive surface samples), however, this was likely due to sample timing as these studies were completed early in the pandemic (April 2020) compared to ours.[9] Cheng et al. performed a similar study of fomites in inpatient hospital rooms housing COVID-19 positive patients and found similar RT-PCR results to our study: 5.0% of all samples were positive compared to our 5.5%, and, among shared study fomites, the bed rails were most likely to be contaminated at 5.4% compared to our 9.2%.[10] Our RT-PCR results and those just cited differed from some other studies such as Zhou et at. (52.3% of surface sample positive); likely because this study was conducted early in the pandemic prior to availability of therapeutic agents.

Few studies have examined environmental contamination using cell culture techniques.[7] Wang et al. did not find SARS-CoV-2 RNA or infectious virus in any environmental samples in a Chinese hospitals' isolation ward. Our cell culture results match prior studies that deployed RT-PCR and cell culture concurrently with all studies reporting higher contamination rates with RT-PCR than cell culture. However, unlike our study most studies did not demonstrate any positive cell culture samples including Colaneri et al. (26 environmental samples, healthcare emergency unit), Wang et al. (36 samples, of isolation wards), and Zhou et al. (218 samples, acute healthcare settings).[3,8,9] However, Santarpia et al. (163 samples,COVID-19 patient isolation rooms) found some evidence of intact SARS-CoV-2 virions in cell culture but did not observe CPE.[11] In summary, our data adds to published literature demonstrating that viable virus uncommonly contaminates room surfaces housing COVID-19 patients.

Our study has several limitations. Patients were potentially later in their disease since timing of hospital presentation and admission does not necessarily reflect timing of infection. The CDC states that infectious virus is not typically shed after day 7 of symptom onset and it is possible our data included patients who were close to, or past, that day.[12] We attempted to control for this by enrolling patients within 24 hours of their first positive SARS-CoV-2 test, however, patients could have been symptomatic days before. This study was also completed in an acute healthcare setting in a COVID-19 specific unit so these results are not generalizable to other healthcare environments such as emergency departments, non-COVID-19 units or outside of the healthcare setting. Lastly, this study has a relatively small sample size and patients were not selected randomly.

In conclusion, our results suggest that RT-PCR inflates the SARS-CoV-2 contamination rate of the healthcare environment and does not indicate the presence of live infectious virus. Importantly, even the detection of live infectious virus via cell culture does not indicate that an infectious does of SARS-CoV-2 is present. More studies including RT-PCR and viral cell culture assays are needed to determine the importance of discovering SARS-CoV-2 RNA versus infectious virus in the clinical environment.

Acknowledgements

Financial Support

This work was supported by a grant from the Duke-UNC Prevention Epicenter Program for Prevention of Healthcare-Associated Infections - Coronavirus Preparedness and Response Supplemental Appropriations Act (grant no. U54CK000483)

Potential Conflicts of Interest

All authors report no conflict of interest with this work. DA reports grant to institution during the conduct of the study from CDC Prevention Epicenter Program; grant to institution from NIH/NIAID and AHRQ outside of the conduct of the study and contract to institution from CDC outside of the conduct of the study; royalties for authorship from UpToDate, Online; and owner of Infection Control Education for Major Sports, LLC.

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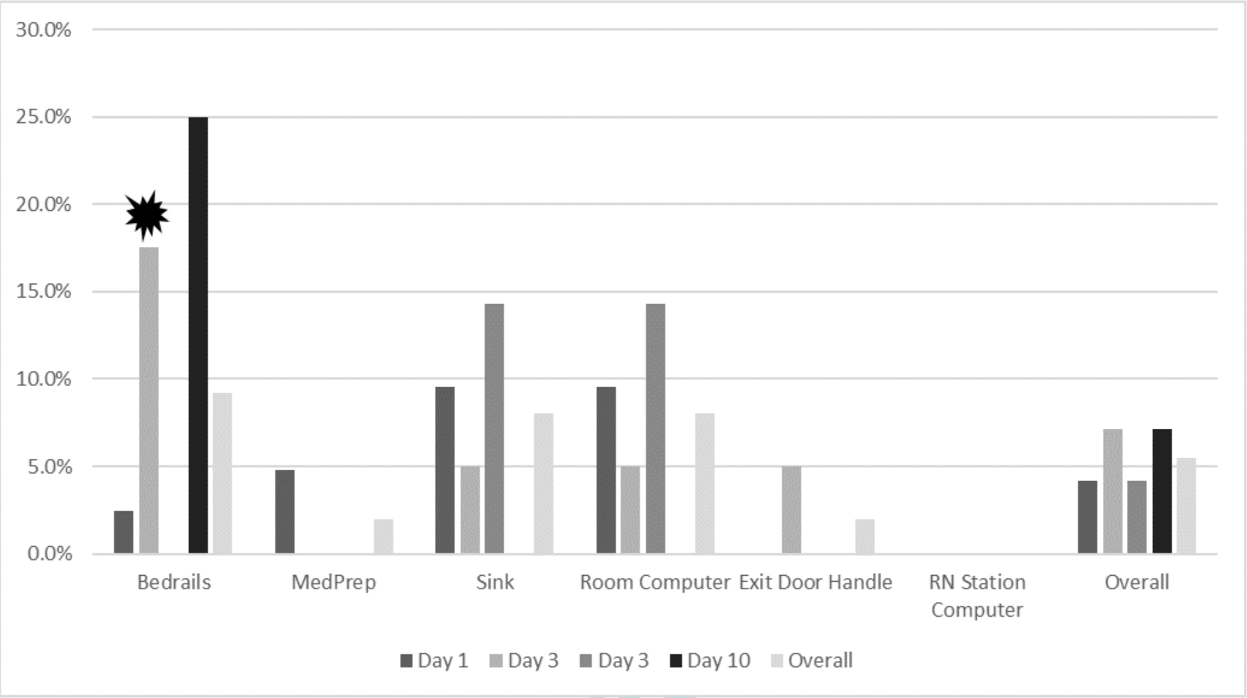
Figure legend:

Figure 1. Proportion of SARS-CoV-2 Positive Environmental Samples by Sample Location and Day

Asterisk Indicates positive cell culture sample

Figure 1


30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Bedrails
MedPrep
Sink
Room Computer
Exit Door Handle
RN Station Computer
Overall
■ Day 1
■ Day 3
■ Day 3
■ Day 10
■ Overall