Measuring SARS-CoV-2 aerosolization in rooms of hospitalized patients

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Abstract
Objective: Airborne spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a significant risk for healthcare workers. Understanding transmission of SARS-CoV-2 in the hospital could help minimize nosocomial infection. The objective of this pilot study was to measure aerosolization of SARS-CoV-2 in the hospital rooms of COVID-19 patients.

Methods: Two air samplers (Inspirotec) were placed 1 and 4 m away from adults with SARS-CoV-2 infection hospitalized at an urban, academic tertiary care center from June to October 2020. Airborne SARS-CoV-2 concentration was measured by quantitative reverse transcription polymerase chain reaction and analyzed by clinical parameters and patient demographics.

Results: Thirteen patients with COVID-19 (eight females [61.5%], median age: 57 years old, range 25–82) presented with shortness of breath (100%), cough (38.5%) and fever (15.4%). Respiratory therapy during air sampling varied: mechanical ventilation via endotracheal tube (n = 3), high flow nasal cannula (n = 4), nasal cannula (n = 4), respiratory helmet (n = 1), and room air (n = 1). SARS-CoV-2 RNA was

Savaş Tay and Jayant M. Pinto have contributed equally to this study.

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identified in rooms of three out of three intubated patients compared with one out of 10 of the non-intubated patients \( (p = .014) \). Airborne SARS-CoV-2 tended to decrease with distance (1 vs. 4 m) in rooms of intubated patients.

**Conclusions:** Hospital rooms of intubated patients had higher levels of aerosolized SARS-CoV-2, consistent with increased aerosolization of virus in patients with severe disease or treatment with positive pressure ventilation through an endotracheal tube. While preliminary, these data have safety implications for health care workers and design of protective measures in the hospital.

**Level of Evidence:** 2

**KEYWORDS**

airway management, COVID-19, infectious disease transmission, intubation

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1 | INTRODUCTION

The ongoing COVID-19 pandemic has resulted in over 500 million reported cases worldwide as of May 1, 2022.\(^1\) Among other high-risk populations, healthcare workers (HCWs) are vulnerable to infection due to direct occupational exposure to infected patients. Early estimates suggested that 10–20% of all diagnoses were in HCWs\(^2\) and nosocomial infection poses a significant and continuing threat. While many HCWs are protected by vaccination, the efficacy of SARS-CoV-2 vaccinations is not 100% and studies have demonstrated symptomatic breakthrough infections in fully vaccinated HCWs.\(^3,4\)

Evidence supports transmission of SARS-CoV-2 through the air, although controversies over parameters that govern spread remain. Studies have linked the spread of the virus to prolonged exposure in poorly ventilated enclosures during aerosol generating activities, such as eating, talking, singing, and exercising, despite adequate distancing and mask use.\(^5,6,7\) Furthermore, in July 2020, the WHO formally acknowledged aerosol transmission of SARS-CoV-2 in many non-hospital settings. Infection control guidelines from the CDC recommend airborne precautions, such as N95 respirators, during aerosol generating procedures (AGPs), but do not consider the presence of aerosols in patient care outside of AGPs.\(^8\) In addition, the vast majority of the literature regarding particle dispersion of SARS-CoV-2 in healthcare settings is centered around AGPs. For instance, aerosol generation has been demonstrated in simulated environments surrounding tracheostomy care,\(^9\) but not office laryngoscopy.\(^10\) Our focus is the possibility of aerosolization of the virus beyond the setting of AGPs, which has major implications for optimal infection control not only in otolaryngology but in a variety of medical disciplines.

Early data show that SARS-CoV-2 may be present in aerosols in different hospital settings outside of AGPs: in the ICU, general COVID-19 wards, bathrooms, hallways, and quarantine units.\(^11,12\) In addition, Lednicky et al. used a cell culture system to demonstrate viability of this aerosolized virus in patient rooms.\(^13\) Karan et al. documented transmission of SARS-CoV-2 to hospital roommates of asymptomatic patients.\(^14\) However, other studies have failed to detect aerosolized SARS-CoV-2 in patient rooms.\(^15,16\) Although heterogeneity in air sampling methods makes direct comparison challenging, these studies suggest that aerosolized SARS-CoV-2 could result in viral transmission in healthcare settings.

How disease status and treatment contribute to aerosolization of SARS-CoV-2 have not been well-characterized. Santarpia et al. detected viral RNA in the air around both asymptomatic and mildly symptomatic quarantined patients on day 6–8 of admission,\(^17\) but did not address other clinical parameters. Moore et al. described the aerosolization of virus 1 day after diagnosis near a patient receiving oxygen by Venturi mask.\(^18\) Lei et al. described positive air samples in an ICU ward housing patients with long term SARS-CoV-2 infection.\(^19\) Other studies detected SARS-CoV-2 in isolation rooms of recovering patients.\(^20,21\) Beyond these data, our understanding of airborne transmission of SARS-CoV-2 in the context of patient respiratory treatment remains limited.

In this pilot study, we used novel air sampling technology to sample air from the rooms of patients hospitalized due to confirmed SARS-CoV-2. Specifically, we compared aerosolized SARS-CoV-2 concentration in patients with various types of respiratory support and determined correlations between aerosolized SARS-CoV-2 concentration and other patient factors. Our goal was to improve evidence-based strategies to protect our health care teams.

2 | MATERIALS AND METHODS

2.1 | Patient recruitment and consent

Newly diagnosed patients with SARS-CoV-2 (by RT-qPCR assay from a flocked nasopharyngeal swab assayed in our hospital’s clinical laboratory using FDA approved methods) at an urban, academic, and tertiary care center were enrolled. We included adults \( \geq 18 \) years of age with standard COVID-19 respiratory symptoms (e.g., rhinorrhea, sneezing, nasal congestion, sore throat, cough, shortness of breath) who were admitted to private, negative pressure inpatient rooms located in the Center for Care and Discovery (CCD) at The University of Chicago Medicine, built in 2013. A total of 13 patients were...
recruited (Table 1). The median age was 57, ranging from 25 to 82 years old. Eight patients were female (61.5%) and five were male (38.5%). Air samplers were deployed within 48 h after patient admission to their inpatient room.

Written, informed consent was obtained immediately prior to placement of the air sampler devices by the patient or by proxy as determined by the Illinois Healthcare Surrogate Act. The study protocol was approved by the University of Chicago Biological Sciences Division Institutional Review Board and the Office of Research Safety.

### 2.2 COVID-19 therapy

Patients were placed on respiratory support to titrate oxygen saturations to SpO2 > 92% and monitored for respiratory symptoms. A rapidly rising oxygen requirement, defined as a need for >6 L of supplemental oxygen, work of breathing not alleviated by current supplemental oxygen, and/or signs of shock or hemodynamic instability, prompted transfer to the intensive care unit (ICU). All non-intubated patients were given an incentive spirometer and instructed on its use. Prone position was standard of care and performed as tolerated, regardless of respiratory support.

During the study period, remdesivir was considered as an adjunctive treatment for patients with a COVID-19 diagnosis within the last 10 days, SpO2 < 94% on room air or need for supplemental oxygen, ALT<10 times the upper limit of normal or chest radiograph with pulmonary infiltrates. Dexamethasone was considered for all patients requiring high flow nasal cannula (HFNC), non-invasive positive pressure ventilation or mechanical ventilation.

### 2.3 Hospital room characteristics

All COVID-19 patients were roomed in standardized negative pressure patient rooms which were equipped with a separate ante room containing a sliding door that must be closed prior to opening the main room door. Each room (Figure 1) had a ventilation in port and out port located on the ceiling, a counter with a sink located opposite the patient’s head of bed, 4 m from the head of the patient, a bedside table, located <1 m from the head of the patient, a television with a portable remote control, a chair, and a hospital computer. Finally, each room was equipped with a bathroom containing a shower and a toilet.

### 2.4 Demographic and clinical information

Demographic and clinical data were collected from the electronic health record. Intubated patients received mechanical ventilation through an endotracheal tube on a closed circuit with in-line suction connected to a wall-mounted suction canister during the air sampling period. Only closed-circuit suctioning was performed. Non-intubated patients were supported on room air, nasal cannula (NC), HFNC or

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**Table 1** Clinical characteristics of study cohort.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Oxygen therapy</th>
<th>Cough</th>
<th>Other symptoms</th>
<th>Age/sex</th>
<th>Day of illness</th>
<th>Airborne viral concentration (copies/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sampler 1 (1 m)</td>
</tr>
<tr>
<td>1</td>
<td>Intubated</td>
<td>No</td>
<td>SOB, fever, nausea</td>
<td>25/F</td>
<td>6</td>
<td>46.04</td>
</tr>
<tr>
<td>2</td>
<td>Intubated</td>
<td>No</td>
<td>Hypoxia, seizure, fever, lethargy</td>
<td>44/F</td>
<td>12</td>
<td>62.59</td>
</tr>
<tr>
<td>3</td>
<td>Intubated—high flow nasal cannula&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>DOE, diarrhea, oliguria, anorexia, sweating</td>
<td>50/M</td>
<td>10</td>
<td>86.33</td>
</tr>
<tr>
<td>4</td>
<td>Respiratory helmet—high flow nasal cannula&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No</td>
<td>SOB, confusion</td>
<td>59/F</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>High flow nasal cannula</td>
<td>Yes</td>
<td>SOB</td>
<td>38/M</td>
<td>12</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>High flow nasal cannula</td>
<td>No</td>
<td>SOB, fatigue, diarrhea</td>
<td>75/F</td>
<td>11</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>High flow nasal cannula</td>
<td>No</td>
<td>SOB</td>
<td>28/M</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>High flow nasal cannula</td>
<td>Yes</td>
<td>SOB, chest tightness, fever, chills, malaise</td>
<td>57/F</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>Nasal cannula</td>
<td>No</td>
<td>SOB, chest pain</td>
<td>59/F</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Nasal cannula</td>
<td>Yes</td>
<td>SOB, lethargy</td>
<td>57/M</td>
<td>14</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>Nasal cannula</td>
<td>No</td>
<td>SOB, loss of taste and smell, diarrhea, myalgias, chills</td>
<td>34/M</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>Nasal cannula</td>
<td>Yes</td>
<td>Weakness</td>
<td>82/F</td>
<td>8</td>
<td>2.878</td>
</tr>
<tr>
<td>13</td>
<td>Room air</td>
<td>No</td>
<td>SOB, taste changes, diarrhea, myalgia, fatigue</td>
<td>77/F</td>
<td>15</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: “–” indicates that airborne viral concentration is non-detectable.

<sup>a</sup> patient was transitioned from endotracheal intubation to high flow nasal cannula on day 11 of illness.

<sup>b</sup> patient was transitioned from a respiratory helmet to high flow nasal cannula on day 3 of illness.
respiratory helmet for the full air sampling period. Patients with tracheostomies were excluded from the study as were those who were discharged or transferred during the sampling period.

2.5 | Air sample collection

The “Exhale” device (Inspiretec Inc., North Chicago, IL) was used to sample air at a rate of >50 L per minute. This is a device that is normally used to sample air for household allergens. The device collects particles as small as nucleic acids and proteins from air. Collection devices were placed in two standardized locations within each patient room; on the patient’s bedside table near the upper airway (1 m distance) and on the counter opposite the foot of the patient’s bed (4 m distance) (Figure 1). The near air sampler presumably assessed both droplet and close-range aerosol transmission of the SARS-CoV-2 virus and the far air sampler measured aerosol transmission only (>6 feet away, the standard CDC social distancing guideline). Air was sampled for 3 days ± 12 h, constrained by safety concerns regarding retrieval early in the pandemic. Air from patient rooms was considered positive for SARS-CoV-2 if either sampler (near or far) tested positive for viral RNA (see below). Patients and providers were instructed to behave as they normally would as if the sample collection device was not in their room, and patients were instructed not to purposely cough, sneeze or speak directly into the collection device. Visitors were not allowed into patient rooms in accordance with hospital policy.

We also sampled the air from rooms of two patients in the cardiothoracic ICU who tested negative for SARS-CoV-2 by nasopharyngeal swab testing as negative controls. One of these patients was intubated throughout the air sampling period and the other patient was treated with HFNC.

2.6 | Viral extraction, amplification, and quantitation

After collection, devices were immediately transported to a BSL2+ facility on campus for viral extraction to perform RNA isolation within 2 h. Collection electrodes were removed from the device and placed in a 15 ml conical tube inside a BSL2 hood. Particles were eluted from these electrodes by gently vortexing the electrodes in 1 ml of nuclease-free water. Then, 139 μl of the eluant was spiked with 1 μl of synthetic HIV-1 RNA (VR-3245SD, ATCC; 0.1× stock concentration) for quality control. Extraction was performed with QIaamp viral RNA mini kit (QIAGEN) following manufacturer’s protocol. RNA samples were stored at −80°C until the RT-qPCR assay (performed within 1 week after the extraction). RT-qPCR was performed in duplicates on each individual sample using CDC recommended SARS-CoV-2 N1 and N2 primers/probes, and HIV primers/probes for RNA extraction quality control. A standard curve with SARS-CoV-2 standards (#COV019, Bio-Rad, Hercules, CA) was performed on every plate for calculation of SARS-CoV-2 concentrations. Concentrations presented represent an average of two RT-qPCR replicates. A sampler was considered positive if successful amplification (Ct ≤ 40) was achieved for at least one replicate for both N1 and N2 probes. SARS-CoV-2 concentrations in copies/ml generated from raw PCR Ct data were calculated and compared.

2.7 | Statistical analysis

We analyzed the significance of the percentage of positive patients by intubation status (Figure 2A) and by presentation with cough (Figure 2B) using 2 × 2 contingency tables. A two-tailed Fisher’s exact test was used to test these associations, with a p value ≤ 0.05 considered statistically significant. A Mann-Whitney U test was used to determine significance between viral concentrations by intubation status (Figure 3A) and by presentation with cough (Figure 3B). A two-tailed Wilcoxon signed-rank test was used to determine significance in paired comparisons of airborne viral concentration at two sampler distances (1 vs. 4 m), with a p value ≤ 0.05 considered as statistically significant. Prism GraphPad V7 software was used for data analysis.
3 | RESULTS

3.1 | Demographics

Material from the air of the hospital rooms from a total of 13 patients was collected between June and October 2020 (patient demographics, Table 1). All patients presented with shortness of breath. Other common presenting symptoms on admission included cough (38.5%), fever (15.4%), diarrhea (30.8%), and fatigue/lethargy (38.5%). Eight patients (61.5%) were African-American, three were Hispanic, one was Caucasian, and one declined to report race; this breakdown was consistent with local demographics. Samplers were deployed within 2 days of patient admission, which corresponded to day one through day 15 of illness by history. There was no correlation between sampler positivity and day of illness.

3.2 | Air samplers

Air from the hospital rooms of four of the 13 patients had detectable SARS-CoV-2 viral RNA (30.8%). Two of the rooms had one sampler and two of the rooms had two samplers containing detectable SARS-CoV-2 RNA. Both COVID-19 negative control patients showed no detectable SARS-CoV-2 RNA in the air from their hospital rooms.

Patients had a range of respiratory interventions, including no respiratory support (1 patient), NC (four patients), HFNC (four patients), respiratory helmet (one patient) and mechanical ventilation with intubation (three patients). Two patients had transitions in respiratory treatment during the air sampling period as indicated in Table 1. The primary clinical factor that determined detectable airborne SARS-CoV-2 RNA was mechanical ventilation with endotracheal intubation (Figures 2A and 3A). Three out of three rooms containing intubated patients compared with only one out of 10 patient rooms with non-intubated patients were found to have detectable SARS-CoV-2 (p = .014). We note that air samplers were placed at least 24 h after the intubation process, and placement of the endotracheal tube occurred in a different location prior to transport to the room where the air was sampled for all three of these patients. Airborne viral concentrations detected via RT-qPCR ranged from 4.32 to 46.04 copies/ml eluant (Table 1).

Presence of detectable viral RNA did not appear to vary by age, gender or race, nor with presentation with cough as described in the admission history and physical examination (Figures 2B and 3B). For these analyses, numbers were too small for definitive determinations.

We found a higher airborne viral concentration at 1 m compared with...
Consistent with this finding, bi-level positive airway pressure (BiPAP) has been associated with increased risk of SARS-CoV-1 disease, such patients may carry a higher viral load in their upper airways, leading to higher levels being emitted into the room. Unfortunately, we do not have data on the viral load present in the upper airway of the patients in this study, so we are unable to perform correlations between aerosolized SARS-CoV-2 and the amount of the virus present in the nasopharynx, sputum, saliva or otherwise at this time. Correlating patient viral load and aerosolized viral concentration in this fashion would be a key question for future studies.

Data on detection of aerosolized SARS-CoV-2 in rooms of intubated patients are sparse and conflicting. For instance, Ahn et al. found viral contamination on the surface of one intubated patient’s endotracheal tube, but not in the air of this patient’s room after 20 min of sampling. Razzini et al. detected aerosolized virus in an open ward housing three patients (two intubated and one non-intubated). While high levels of SARS-CoV-2 viral material on surfaces has been detected in intubated patient rooms, a lack of equipment has inhibited further investigation of aerosolization outside of simulated environments or in non-infected subjects. The ability to detect viral RNA in the current study may be due to the increased sensitivity of the air sampling technology we employed and extended length of our sampling (3 days). Additionally, for the first time, we compared detection of aerosolized SARS-CoV-2 between intubated and non-intubated patients to distinguish if respiratory status was associated with higher levels of virus.

There were several limitations to this study. We had a very limited sample size due to the constraints of performing research early in the ongoing pandemic and the change in treatment algorithms toward avoiding intubation. This sample size precluded meaningful subgroup analyses or testing of specific clinical parameters, or adjusted analyses. Additionally, we have no direct data on whether aerosolized viral RNA results in viable viral particles and thus we cannot make definitive conclusions regarding risk of transmission to HCWs. Interestingly, inert, culture-negative virus can give rise to positive tests. While we did not find any correlation between day of hospitalization and viral aerosolization, it should be noted that the risk of infectivity is highest earlier in the course of the illness, which has informed the course of clinical care. The confounding of intubation/mechanical ventilation and disease severity preclude the ability to determine which of these was independently associated with viral aerosolization. Future work in larger cohorts is necessary to address these issues.
Air sampling has been performed to detect the SARS-CoV-1 virus, as well as more common respiratory viruses, such as influenza A, RSV, and adenovirus successfully.\textsuperscript{12,39-41}

The technology we employed is novel and has been used to detect other viral pathogens (e.g., Venezuelan equine encephalitis virus\textsuperscript{42}), but has not been compared with other air sampling methods. Therefore, the exact threshold of airborne SARS-CoV-2 correlating with a negative result in our air samplers is not known. When calculating the number of viral particles per liter air in the room from the calculated airborne viral concentration, we find a range from 1 viral particle per 8500 to 1 per 45,000 L of air, even for near samplers that would potentially detect droplet transmission of SARS-CoV-2 (Table 2). This may reflect detection performance or low density of viral particles. Thus, the inability to detect SARS-CoV-2 in non-intubated patients does not definitely rule out aerosolization in this setting. Nevertheless, these data are compelling enough to justify future work at a larger scale to inform the question of airborne spread of SARS-CoV-2 in the hospital. To our knowledge, none of the health care team members caring for these patients contracted COVID-19.

Although it was not statistically significant, our finding that airborne viral concentration may be higher at 1 versus 4 m of distance from the upper airway of these patients is intriguing. The question of distance is paramount, not only inside the hospital, but indoors more generally as well as in public settings. Testing this question to tease out the relationship between viral aerosolization and distance is important, if challenging, next step. In addition, air sampling during known AGPs would allow us to quantify increases in aerosolized virus caused by these procedures and assess risk of other methods of respiratory support and devices, such as tracheostomies.

These data have important implications for HCW safety protocols, especially in the intensive care setting. The proportion of COVID-19 inpatients requiring mechanical ventilation have remained relatively constant following an initial peak in first few months of the pandemic.\textsuperscript{43} As such, HCWs will continue to be exposed to intubated patients as the pandemic continues. Our study demonstrates that nurses, physicians, speech language pathologists, respiratory care practitioners, and other team members may face higher risks in caring for COVID-19 patients who are intubated and precautions should be taken to minimize virus emission through closed circuits. Care should be taken to minimize exposure time closer to the patient’s airway as that zone may have higher amounts of aerosolized virus. Overall, HCWs should pursue a high degree of vigilance when caring for intubated patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Oxygen therapy</th>
<th>Cough</th>
<th>L air/viral particle (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 m</td>
</tr>
<tr>
<td>1</td>
<td>Intubated</td>
<td>No</td>
<td>6192.45</td>
</tr>
<tr>
<td>2</td>
<td>Intubated</td>
<td>No</td>
<td>6902.07</td>
</tr>
<tr>
<td>3</td>
<td>Intubated—high flow nasal cannula\textsuperscript{a}</td>
<td>Yes</td>
<td>4553.64</td>
</tr>
<tr>
<td>4</td>
<td>Respiratory helmet—high flow nasal cannula\textsuperscript{b}</td>
<td>No</td>
<td>●</td>
</tr>
<tr>
<td>5</td>
<td>High flow nasal cannula</td>
<td>Yes</td>
<td>●</td>
</tr>
<tr>
<td>6</td>
<td>High flow nasal cannula</td>
<td>No</td>
<td>●</td>
</tr>
<tr>
<td>7</td>
<td>High flow nasal cannula</td>
<td>No</td>
<td>●</td>
</tr>
<tr>
<td>8</td>
<td>High flow nasal cannula</td>
<td>Yes</td>
<td>●</td>
</tr>
<tr>
<td>9</td>
<td>Nasal cannula</td>
<td>Yes</td>
<td>130604.4</td>
</tr>
<tr>
<td>10</td>
<td>Nasal cannula</td>
<td>No</td>
<td>●</td>
</tr>
<tr>
<td>11</td>
<td>Nasal cannula</td>
<td>Yes</td>
<td>●</td>
</tr>
<tr>
<td>12</td>
<td>Nasal cannula</td>
<td>No</td>
<td>●</td>
</tr>
<tr>
<td>13</td>
<td>Room air</td>
<td>No</td>
<td>●</td>
</tr>
</tbody>
</table>

Note: “…” no sampler was deployed. “●” airborne viral concentration was non-detectable, and L air/viral particle was non-calculable.

\textsuperscript{a}Patient was transitioned from endotracheal intubation to high flow nasal cannula at 24 h.
\textsuperscript{b}Patient was transitioned from a respiratory helmet to high flow nasal cannula at 48 h.

5 | CONCLUSION

Higher levels of SARS-CoV-2 are present in the air of hospital rooms of intubated COVID-19 patients, consistent with the hypotheses of increased aerosolized SARS-CoV-2 in critically ill patients or increased particle dispersion secondary to positive pressure ventilation. Our study highlights the need for adequate precautions to be taken by HCWs caring for any intubated COVID-19 patient. Further studies are needed to determine the viability of virus in the air of rooms containing COVID-19 patients, further evaluate the relationship between viral aerosolization and distance, and assess patient and clinical parameters that are associated with increased viral aerosolization. Extension of this approach of measuring SARS-CoV-2 to other pathogens may result in improved public health worldwide for other, similar respiratory diseases.
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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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REFERENCES


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