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Levels and particle size distribution of airborne SARS-CoV-2 at a healthcare facility in Kuwait

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HIGHLIGHTS
• Airborne SARS-CoV-2 was found in many indoor and outdoor locations in Jaber Hospital.
• Concentrations were highest in symptomatic patient rooms.
• SARS-CoV-2 RNA was found on large particles (>10 μm) in symptomatic patient rooms.
• SARS-CoV-2 RNA was detected in smaller particles (<10 μm) in ICUs and outside.
• Fine particles with SARS-CoV-2 suggests that the virus can travel farther than 1 m.

GRAPHICAL ABSTRACT

ABSTRACT

The Coronavirus Disease 2019 (COVID-19) pandemic spread rapidly despite extraordinary screening and social distancing measures. Such rapid spread was due in part to the fact that the disease transmission, particularly via airborne spread, is poorly understood. Characterizing the airborne size distribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential to understanding the risk of airborne transmission. We collected size-fractionated (<2.5, 2.5–10, and ≥10 μm) samples using a cascade impactor at more than 30 locations inside and outside Jaber Hospital and the nearby Temporary Quarantine Facility in Kuwait from April to July 2020. We hypothesized that airborne SARS-CoV-2 would be present in all size fractions, including fine particles, and in a size distribution that differed by sampling location. We found that 6% of the samples (13 out of 210) were positive for SARS-CoV-2 RNA. Concentrations ranged from 3 to 25 copies/m3. The size distribution of particle-associated SARS-CoV-2 was different for each location. Large (>10 μm) particles with the virus were found in symptomatic patient rooms. Fine (<2.5 μm) particle-associated SARS-CoV-2 was detected in rooms with intubated patients and outside the hospital entrance gates. Coarse (2.5–10 μm) virus-laden particles were present in all locations with positive samples. This is one of the most comprehensive studies to date on size-fractionated airborne SARS-CoV-2 RNA. Our findings support location-specific precautions that mitigate the spread of particles including fine particulate matter over distances greater than 1 m, including in locations outside the hospital.

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**Abbreviations:** COVID-19, coronavirus disease 2019; GFF, glass fiber filter; ICU, intensive care unit; PM, particulate matter; PPE, personal protective equipment; PUF, polyurethane foam; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TSP, total suspended particulate.
1. Introduction

Coronavirus Disease 2019 (COVID-19) spread rapidly around the world and challenged existing conceptions of disease transmission and prevention (Bourouiba, 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, was recently shown to remain viable in aerosols for up to 16 h (Van Doremalen et al., 2020; Smith et al., 2020; Fears et al., 2020). However, little is known about the size distribution of SARS-CoV-2 in indoor and outdoor air. This property of airborne SARS-CoV-2 is essential for predicting the spread of the disease.

Of particular concern, healthcare workers are at increased risk of occupational exposure to SARS-CoV-2. The U.S. Occupational Safety and Health Administration (OSHA) now recommends airborne precautions to protect healthcare workers with exposure to the virus (U.S. Department of Labor, 2020). While many hospitals around the world grappled with risk reduction strategies for frontline healthcare workers during the pandemic, there is also notable nosocomial COVID-19 transmission among inpatients (Rhee et al., 2020). Infection control strategies aim to curb such risks as well as improve the resilience of healthcare workers. It becomes imperative to understand the role of air particles that serve as vectors for SARS-CoV-2 in the hospital workplace in which conventional infection control strategies may fail short.

Traditionally, the medical community has relied on the dichotomy of droplet (large particles that infect behave like projectiles) and aerosol (smaller particles that remain airborne for longer periods and can be inhaled) to characterize host-to-host transmission mechanisms. This is essential for risk management and allocation of resources to control respiratory infectious diseases (World Health Organization, 2014). In the COVID-19 pandemic, authorities stressed the importance of droplet transmission. However, breathing, talking, singing, and coughing release particles of a continuum of sizes (Johnson et al., 2021). Even larger particles that are produced from processes such as coughing (Yang et al., 2007) and sneezing (Bourouiba, 2014) may dry out and become smaller (Wells, 1934). Evidence suggests that smaller aerosols, including those generated by speaking, may be a vector for COVID-19 (Morawska and Milton, 2020; Morawska and Cao, 2020; Noormotlagh et al., 2020; Stadnytskyi et al., 2020). These smaller particles have significant health impacts, as they are capable of penetrating deeper into the lungs. In other respiratory diseases, pathogens on smaller particles require fewer microorganisms to cause an infection than those on larger particles (Thomas, 2013; Nicas et al., 2005; Tellier, 2006). A wealth of air pollution studies have shown that fine particulate matter (≤2.5 μm) remains airborne for several hours before being removed from the air by gravitational settling (Wells, 1934; Hinds, 2012; Liu et al., 2018; Xie et al., 2020; Balh et al., 2020). As a result, smaller particles can travel farther than 1 m, which is the social distancing space currently recommended by the World Health Organization (World Health Organization, 2020), and have a greater opportunity to infect someone who crosses their path. Information about the size distribution of airborne particles carrying SARS-CoV-2 is useful to model expected transmission distance and establish maximum coverage by airborne precautions.

Despite the important implications of the SARS-CoV-2 size distribution, there is little information about its presence with airborne size-specific particles in indoor and outdoor locations. Recent studies are extremely limited in scope. Early in the pandemic, Liu et al. (2020) and Chia et al. (2020) collected three times each in healthcare settings. Lednicky et al. (2021) examined aerosols inside a car driven by a patient with COVID-19, with one collection in five individual size fractions. Santarpia et al. (2020a) collected aerosols inside the rooms of COVID-19 patients six times, for a total of 18 individual size-fractionated samples. Stern et al. (2021) collected 30 times in three size fractions inside a U.S. hospital. There are still unanswered questions about the severity of risk of small particle-associated COVID-19 transmission and the likelihood of location-dependent viral RNA concentrations in the air.

In this over two-month-long study, we present the most comprehensive COVID-19 air-sampling program in healthcare workplaces to date, with 70 collections and 210 individually analyzed size-fractionated samples. We collected simultaneous air samples in three different size fractions at over 30 hospital locations including intensive care units (ICUs), nurses’ workstations, the rooms of inpatients with and without symptoms, observation rooms (transitional treatment units in the Emergency Department where patients are continuously evaluated before the decision to admit or discharge), locker rooms, bathrooms, a lobby, waiting areas, patient hallways, swab testing areas, and outside hospital entrances. We examined the size distribution of SARS-CoV-2 in airborne particles in three distinct size fractions: fine particles (≤2.5 μm) (which are small enough to remain suspended in the air and penetrate deep into the airways and directly into the bloodstream), coarse particles (2.5–10 μm) (which are inhalable but do not penetrate deeply into the lungs), and large particles (≥10 μm) (which have relatively high settling velocities and travel shorter distances before settling out of the air). We hypothesized that SARS-CoV-2 RNA would be present in indoor air in different size fractions, including fine particulate matter, and that the aerodynamic size distribution would depend on the sampling location.

2. Methods

2.1. Site

Jaber Hospital is located in South Surra, Kuwait (29°16′34.7″N, 48°00′56.1″E) and was opened in 2018. The hospital area is 695,000 m² with 13 floors and two underground floors. The hospital houses 1168 beds. The Temporary Quarantine Facility (TQF) is a separate building that is located in front of Jaber Hospital and was used to support the Ministry of Health when Jaber Hospital was fully occupied. The TQF has four floors comprising a total area of 12,531 m² and 85 patient rooms. During the sampling period, the TQF was used for swab testing and as a temporary location for COVID-19 patients.

In this study, Jaber Hospital and the TQF were the sole designated COVID-19 facilities in Kuwait. The Jaber Hospital only treated COVID-19 patients (patients with other ailments were treated elsewhere). All patients who had a positive reverse transcription polymerase chain reaction (RT-PCR) test were admitted to the hospital for quarantine, observation, and treatment. Because of this admission policy, even asymptomatic patients (those who tested positive but did not show any symptoms) were admitted to the hospital. Asymptomatic patients were discharged from the hospital only when they repeatedly tested negative on nasopharyngeal swabs.

2.2. Sample locations

We conducted two sampling campaigns. The first campaign lasted from April 30 to May 20, 2020. The second campaign occurred between June 24 and July 10, 2020. The first campaign was only conducted at Jaber Hospital (inside and outside). Locations in the first campaign included ambient-pressure ICUs, inside rooms of symptomatic and asymptomatic patients with one to four beds, nearby nurses’ workstations, inside a nurses’ locker room, and at three different outside gates (Table S1). The second campaign included Jaber hospital (inside and outside), as well as locations inside and outside the TQF. Jaber Hospital locations during the second campaign included negative pressure ICUs, ambient pressure ICUs, bathrooms (2 m away from the toilet), observation rooms, and an outdoor gate. Locations at the TQF during the second campaign included a reception waiting area, COVID-19 swab testing waiting area, swab testing area exit, patient area hallway, and main entrance gate (Table S2). We collected 84 samples in the first campaign from Jaber Hospital locations, 66 samples from the second campaign from Jaber Hospital locations, and 60 samples from the second campaign at the TQF (210 samples). We used a blank cascade for each
collection period (36 total) and processed them simultaneously with the corresponding samples for that given period.

2.3. Sampling

We used custom-designed Harvard Micro-Environmental Cascade Impactors (Demokritou et al., 2002) to collect simultaneous samples in three distinct size fractions: fine ($\leq 2.5 \mu m$ aerodynamic diameter), coarse ($2.5–10 \mu m$), and large ($\geq 10 \mu m$) (Fig. 1). The first impactor stage used a polyurethane foam (PUF) substrate to collect large particles. The second impactor stage used a smaller PUF substrate to collect particles in the coarse size range. The last stage collected fine particles on a 37-mm diameter glass-fiber filter. For each sampling period, the impactor ran continuously for 48 h at a constant flow rate of 5 L/min, which corresponds to a total sampling volume of 14.4 m$^3$.

2.4. Post-sample processing

Following each sample collection, we processed the samplers in a biosafety level-2 laminar flow hood equipped with a high-efficiency particulate air (HEPA) filter. We aseptically removed the samples and transferred them into individual 5-ml sterile centrifuge tubes, submerged the substrates in RNA-later Stabilization Solution (Ambion, Inc., Austin, TX, USA), sealed the tubes with parafilm tape, and placed each tube inside an individual sterile Whirl-Pak (Whirl-Pak®, Nasco, USA) bag. We stored all samples and blanks at 4 °C, and then shipped them overnight on ice to Molecular Research LP (Shallowater, Texas, USA).

2.5. RNA extraction and RT-qPCR

RNA extraction and reverse transcription quantitative polymerase chain reaction (RT-qPCR) were performed at Molecular Research LP (Shallowater, Texas, USA). Viral RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Furthermore, 5.6 μg Poly-A carrier RNA (Qiagen, Hilden, Germany) was also mixed with each sample for extraction. Carrier RNA enhances the low copy viral nucleic acids binding to the mini column. In addition, it reduces the chance of viral RNA degradation. RNA was eluted in 40-μL RNase free water. RNA quantity and quality were determined using NanoDrop2000 (Thermo Scientific, Waltham, MA, USA). Samples were then used to quantify the viral concentrations by qPCR using 2019-nCoV CDC qPCR probe assays (Integrated DNA Technologies, Inc., Coralville, IA, USA) in StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The qPCR reaction was carried out with an initial holding stage of 95 °C for 3 min for PCR enzyme activation. The cycling stage consisted of 45 cycles at 95 °C for 5 s, followed by 55 °C for 30 s. Genomic RNA from SARS-CoV-2 (2019-nCoV/USA-WA1/2020; ATCC, Manassas, VA, USA) was used as a standard. We identified positive samples as those below a cycle threshold of 39.

2.6. Statistical analysis

We calculated the percent of positive samples by dividing the number of positive samples by the number of samples collected for each location category. The air concentrations of SARS-CoV-2 RNA copies are calculated by Eq. (1):

$$C = \frac{p \times 20 \times e_v \div s_v \div v_{air}}{C_2}$$

where $C$ = concentration (copies/m$^3$), $p$ = copies/2 μL cDNA estimated by PCR, $e_v$ = extraction volume (μL), $s_v$ = sample volume of storage liquid (μL), and $v_{air}$ = volume of air collected (m$^3$). The copies/2 μL of cDNA from PCR were estimated using a standard curve. Adjustment by the factor of 20 was used to account for the fact that only 2 μL of cDNA were used out of the total 40 μL elution. We summarize sample concentrations (in copies/m$^3$) using the means and standard deviations of samples collected at any given location.

3. Results

We collected 210 samples during the study period. Out of the Jaber Hospital samples (150 samples total), 13 were positive (Table 1). Among the 13 positive samples, eight were detected at indoor locations and five were detected at the outside locations near the hospital entrance gates. Among the indoor locations, positive samples were observed in the ambient-pressure ICUs (two positive samples) and in the rooms of multiple- and single-bed symptomatic patients (six positive samples). The remaining indoor locations at Jaber Hospital (negative pressure ICU, asymptomatic patient rooms, nurse’s workstations, nurse’s locker room, staff bathrooms, hospital lobby, and observation rooms) had no positive samples. For the outdoor locations, we observed positive samples outside all three hospital entrances (Gates 1, 2, and 7). Gate 1 was used for suspected patients who visited the hospital for COVID-19 testing. Gate 2 was used for medical staff and visitors without symptoms. Gate 7 was used as an entrance for confirmed COVID-19 patients and ambulances bringing confirmed patients for hospitalization.

![Fig. 1. Micro-environmental cascade impactor designed and custom-built by the Environmental Chemistry Laboratory at the Harvard T.H. Chan School of Public Health (HSPH). PUF = polyurethane foam.](image-url)
All 60 samples at the TQF were negative for SARS-CoV-2 RNA (Table S4). All blanks were negative for SARS-CoV-2 RNA.

The fraction of positive samples was greatest for the symptomatic patient rooms (six out of 24 samples or 25%) (Table S5). The ambient-pressure ICU and outside the hospital gates had similar rates of positive samples (two out of 21 or 10% for the ICU; five out of 33 or 15% for outside gates). None of the negative pressure ICU samples were positive.

In Fig. 2, we present average concentrations of SARS-CoV-2 RNA across the location categories. Maximum concentrations in the rooms of symptomatic patients (25 copies/m³) were higher than maximum concentrations in all other sampling locations (17–21 copies/m³) (Fig. 2; Table S4). None of the samples in the rooms of asymptomatic patients were positive. Concentrations in the locations outside the hospital gates (3–17 copies/m³) were relatively low compared to those in the symptomatic patient rooms (8–25 copies/m³) and the ICUs (18–21 copies/m³). Among the outdoor locations, Gate 7 had the highest viral concentration (17 copies/m³ in the fine size fraction).

SARS-CoV-2 RNA was present in all three size fractions examined. The coarse size fraction had more positive samples (seven samples) than the fine (three samples) or large (three samples) sizes. In addition, the size distribution was related to the area in which the sample was collected (Table 1). Positive samples from symptomatic patient rooms were in the coarse (three samples) and large (three samples) size fractions. ICU samples were positive in the fine (one sample) and coarse (one sample) sizes. Outside the hospital gates had positive samples in the fine (two samples) and coarse (three samples) size fractions.

4. Discussion

The results comprise the largest dataset on size-fractionated airborne SARS-CoV-2 RNA to date. We collected 210 size-fractionated samples at Kuwait’s designated COVID-19 inpatient state-of-the-art hospital and quarantine facility. We found positive samples in all three size fractions (fine ≤2.5 μm, coarse 2.5–10 μm, and large ≥10 μm). We confirmed our hypothesis, finding positive samples in the fine size fraction and in a pattern indicating location-specific size distributions.

A key finding is that the size distribution of particle-associated SARS-CoV-2 was related to the location where the particles were collected. Samples collected in symptomatic patient rooms were associated with larger particles, which may be because patients were not required to wear masks at all times in their rooms. Symptomatic patients were also more likely to cough and sneeze, and both of these behaviors generate larger particles than speaking and breathing (Papineni and Rosenthal, 1997). By contrast, all samples collected in the asymptomatic patient rooms were negative. This could be because asymptomatic patients had a longer hospital stay than symptomatic patients and therefore may have become less infectious over time (Wölfel et al., 2020).

This conclusion is also in agreement with Chia et al. (2020), who reported that concentrations of the virus in air samples at hospital settings are highest in the first week of COVID-19 illness (Chia et al., 2020).

Positive samples in the ambient-pressure ICUs were in the fine and coarse sizes but not in the large size fraction, perhaps because these patients were intubated and unable to shed large particles through actions such as coughing and sneezing. Previous research indicates that exhaled particles from mechanically ventilated patients are between 0.3 μm and 1 μm, which is smaller than the large size fraction tested (Wan et al., 2014). This size distribution agrees with our finding of SARS-CoV-2 RNA in the fine size fraction in the intubated patient rooms. It reinforces the importance of airborne personal protective gear for ICU healthcare workers that limits small particle exposure, especially for physicians and nurses attending to intubated patients. The emphasis on “droplet precautions” during patient intubation and extubation leads to an emphasis on large droplet routes but does not account for smaller aerosol precautions that could also prevent exposure (Weissman et al., 2020; Zuo et al., 2020).

None of the negative pressure ICU sampling locations yielded positive results. The negative pressure precautions appeared to be effective at keeping airborne concentrations of the virus low or zero (Smith et al., 2006; Siegel et al., 2007; Lee and Jeong, 2020). Engineering controls such as negative pressure could be challenging in some hospital environments that are already overwhelmed; yet, we show that these conditions appear to be effective.

Outdoor locations are considered to pose reduced risk for COVID-19 transmission compared to indoor locations due to greater dilution and UV exposure (Nishiura et al., 2020; Qian et al., 2020), so it was unexpected to find a similar percent of positive samples in the outside gate locations and the indoor ambient-pressure ICU. Positive samples in the outdoor locations are perhaps due to people nearby these locations being symptomatic, in close proximity, and emitting high viral loads. Positive samples from the outside gates were in the fine and coarse size fractions. Emissions sources at the outside gates may be located farther from the sampler, thus producing particles at the smaller end of the spectrum. In addition, transport processes in outdoor air may select for smaller sizes due to evaporation and settling out of larger particles. Concentrations were higher at the gates with suspected and confirmed patients (Gate 7 and Gate 1) compared to Gate 2, which was designated for staff and visitors without symptoms. Gate 7, which was used for confirmed COVID-19 patients, had an even greater concentration and positive sample rate than Gate 2, which was used for suspected patients. Areas just outside the hospital – which may be thought to have reduced risk of exposure to the virus and where visitors promptly remove their masks – should perhaps be considered part of the hospital with respect to COVID-19 prevention and policies.

Numerous studies have detected SARS-CoV-2 RNA in airborne samples that collected total suspended particulate (TSP) air samples using a single, bulk sample without size fractionation (Santarpia et al., 2020b; Ding et al., 2020; Kenarkooi et al., 2020; Binder et al., 2020; Lednicky et al., 2020; Razzini et al., 2020). Other studies collecting TSP did not find any SARS-CoV-2 RNA at all (Li et al., 2020; Ong et al., 2020; Cheng et al., 2020; Faridi et al., 2020; Masoumbeigi et al., 2020). However, very few studies have collected size-fractionated samples. Liu et al. (2020) and Chia et al. (2020) collected three times each. Santarpia et al. (2020) (Santarpia et al., 2020a) collected six times, Stern et al. (2021) collected 30 times, and Lednicky et al. (2021) collected once. All of these studies found positive samples in multiple size fractions, which agrees with our results. Santarpia et al. (2020a) found SARS-CoV-2 RNA in <1, 1–4, and >4.1 μm sizes. Lednicky et al. found SARS-CoV-2 RNA in all sizes examined except for the smallest one (<0.25 μm) inside a car driven by a COVID-19 patient (Lednicky et al., 2021). Chia et al. found the virus in particles 1–4 μm and >4 μm size fractions inside COVID-19 patient rooms (Chia et al., 2020). Stern et al. (2021) found SARS-CoV-2 RNA in particles <2.5, 2.5–10, and >10 μm inside a hospital.

| Location Category | ≤2.5 μm | 2.5–10 μm | >10 μm | Total |
|-------------------|-------|--------|-------|------|
| ICU – ambient pressure | 1    | 1     | 0     | 2    |
| ICU – negative pressure | 0    | 0     | 0     | 0    |
| Symptomatic patient rooms | 0    | 3     | 3     | 6    |
| Asymptomatic patient rooms | 0    | 0     | 0     | 0    |
| Nurse’s stations | 0    | 0     | 0     | 0    |
| Nurse’s locker room | 0    | 0     | 0     | 0    |
| Observation rooms | 0    | 0     | 0     | 0    |
| Staff bathrooms | 0    | 0     | 0     | 0    |
| Hospital lobby | 0    | 0     | 0     | 0    |
| Outside hospital entrance gates | 2    | 0     | 0     | 2    |
| TQF – outside main gate | 0    | 0     | 0     | 0    |
| TQF – swab exit | 0    | 0     | 0     | 0    |
| TQF – swab waiting area | 0    | 0     | 0     | 0    |
| TQF – reception waiting area | 0    | 0     | 0     | 0    |
| TQF – patient area hallway | 0    | 0     | 0     | 0    |
| Total | 3    | 7     | 3     | 13   |

*TQF = Temporary Quarantine Facility.
Our finding of location-dependent size preferences for SARS-CoV-2 is supported by the results of Liu et al. (2020). The authors found that smaller size fractions were associated with personal protective equipment (PPE) removal rooms and larger sizes were associated with medical staff of fines. However, Liu et al. (2020) found greatest concentrations near the toilet and PPE removal rooms (Liu et al., 2020). By contrast, in the present study, we did not find any virus copies in the staff bathrooms or nurse's locker rooms. It appears that areas of greatest risk for exposure to airborne SARS-CoV-2 RNA may vary by hospital. Alternatively, the differences may simply be due to chance given the low number of positive samples were not sufficient for statistical analysis in either study.

The maximum concentration (25 copies/m³) in our study was lower than those reported by previous studies (42 copies/m³ for Liu et al. (2020), 51 copies/m³ for Stern et al. (2021), and 2000 copies/m³ for Chia et al. (2020)). There are many potential reasons that could explain this. First, there were methodological differences concerning proximity to the sampling device, RNA degradation, extraction efficiency from substrates, and PCR sensitivity. Second, the hospital settings were different. Jaber Hospital is a newly built, state-of-the-art facility that opened in 2018 with spacious rooms and hallways that are continuously disinfected. The hospital had very strict rules about limiting access and foot traffic. In comparison to hospitals in other studies (Liu et al., 2020; Santarpia et al., 2020b; Stern et al., 2021), the Jaber Hospital is very large, which may have
had dilution effects that further reduced levels of airborne virus. In the second campaign, all Jaber Hospital locations were negative, which may be partly explained by the decrease in foot traffic in the hospital, as during this campaign there were additional locations open for COV-19 swab testing and hospitalization to reduce overcrowding. While hospital-specific practices, such as the maximum number of people allowed in the room at once and air exchange rate, may alter the risk of exposure, it is also imperative to acknowledge the potential uncertainty that is associated with the current study design, individual shedding, and location generalizability. Variability in emission rates from sources can vary by orders of magnitude (Ma et al., 2020; Buonanno et al., 2020). Differences in maximum concentration between air sampling studies are likely to be explained by variability in environmental measures as well as individual variability in viral shedding.

This study has some limitations. First, the concentrations estimated in this study were time-weighted averages over 48-h sampling periods, and it is possible that the emissions of the virus into the air occurred in discrete bursts rather than continuously over the entire sampling period. Thus, someone exposed may have received a higher ‘dose’ in the air than those implied based on the measured concentrations. Additionally, we were not able to obtain data on air exchange rates in the indoor sampling locations and therefore we could not assess the effects of ventilation on the presence of airborne SARS-CoV-2 or the number of viral copies in positive samples. The infectious dose of SARS-CoV-2 is still unknown. It is possible that it is similar to that of SARS-CoV-1 (Beggs, 2020), which was estimated to require 280 viral particles to cause illness in 50% of people (Watanabe et al., 2010). Further, given the sampling method, the present study did not measure viability of the virus, which would be necessary to determine whether SARS-CoV-2 is capable of causing infection. Previous studies have found SARS-CoV-2 to be viable in hospital air, including in particles <1 μm (Santarpia et al., 2020a; Lednicky et al., 2020). Finally, because of the low number of positive samples, we were not able to statistically test differences by location or size fractions.

5. Conclusion

While hospitals confront inevitable COVID-19 outbreaks among healthcare workers, a better understanding of the properties of airborne SARS-CoV-2 is essential to predict the spread of the disease in hospital settings. Providing the largest dataset of size-fractionated airborne SARS-CoV-2 RNA to date, we showed that SARS-CoV-2 RNA is present with airborne particles ≥2.5 μm, 2.5–10 μm, and ≥10 μm. The size distribution of the virus depended on the sampling location, which may be due to a combination of varying emission mechanisms, distance from the source, presence of symptomatic patients, and amount of human foot traffic. Hospital staff would benefit from considering location-specific features – including foot traffic, airflow, cleaning frequency, and patient status – when designing airborne prevention protocols. Airborne precautions, including those targeting fine particulate matter, seem to be a convincing strategy to protect healthcare workers from the risk of COVID-19.

Other contributors

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Author access to data

All authors have reviewed and approved the contents of the manuscript and have access to the data used. The data are not identifiable.

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This work and figures have not been published or submitted for consideration elsewhere.

CRediT authorship contribution statement

Rebecca A. Stern: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. Ali Al-Hemoud: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. Barrak Alahmad: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. Petros Koutrakis: Conceptualization, Investigation, Formal analysis, Supervision, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sctotenv.2021.146799.

References

Bahl, P., Doolan, C., de Silva, C., Chughtai, A.A., Bourouiba, L., Machtrey, C.R., 2020. Airborne or droplet precautions for health workers treating Coronavirus Disease 2019? J. Infect. Dis. 1–8 https://doi.org/10.1093/infdis/jiaa189.
Beggs, C.B., 2020. Is there an airborne component to the transmission of COVID-19? a quantitative analysis study. medRxiv 2020.2011.0331.11.20207995 https://doi.org/10.1101/2020.05.22.
Binder, R.A., Alarja, N.A., Robie, E.R., et al., 2020. Environmental and aerosolized severe acute respiratory syndrome coronavirus 2 among hospitalized coronavirus disease 2019 patients. J. Infect. Dis. 222 (11), 1798–1806. https://doi.org/10.1093/infdis/jiaa575.
Bourouiba, L., 2020. Turbulent gas clouds and respiratory pathogens: potential implications for reducing transmission of COVID-19. JAMA 323 (18), 1837–1838. https://doi.org/10.1001/jama.2020.4756.
Bourouiba, L., Dehandschoewercker, E., Bush, J.W., 2014. Violent expiratory events: on coughing and sneezing. J. Fluid Mech. 745, 537–563.
Buonanno, C., Stabile, L., Morawska, L., 2020. Estimation of airborne viral emission: quanta emission rate of SARS-CoV-2 for infection risk assessment. Environ. Int. 141 (May), 105794. https://doi.org/10.1016/j.envint.2020.105794.
Cheng, V.C.C., Wong, S.C., Chan, V.W.M., et al., 2020. Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19). Infect. Control Hosp. Epidemiol. 41 (11), 1258–1265. https://doi.org/10.1017/ice.2020.282.
Chia, P.Y., Coleman, K.K., Tan, Y.K., et al., 2020. Detection of air and surface contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in hospital rooms of infected patients. Nat. Commun. 11 (1), 1–7.
Demokritou, P., Gupta, T., Ferguson, S., Koutrakis, P., 2002. Development and laboratory performance evaluation of a personal cascade impactor. J. Air Waste Manag. Assoc. 52, 1230–1237.
Ding, Z., Qian, H., Xu, B., et al., 2020. Toilets dominate environmental detection of SARS-CoV-2 virus in a hospital. medRxiv https://doi.org/10.1101/2020.04.03.20052175.
Faridi, S.,Naiz, S.,Sadeghi, K.,et al., 2020. A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran. Sci. Total Environ. 725, 138401.
Fears, A., Klimestra, W., Duprex, P., et al., 2020. Comparative dynamic aerosol efficacies of three emergent coronaviruses and the unusual persistence of SARS-CoV-2 in aerosol suspensions. medRxiv Prepr. Serv. Heal. Sci. 2. https://doi.org/10.1101/2020.04.11.20063784.
Hinds, W.C., 2012. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles, John Wiley & Sons.
Johnson, G.R., Morawska, L., Ristovski, Z.D., et al., 2011. Modality of human expired aerosol size distributions. J. Aerosol Sci. 42 (12), 839–851.
Liu, C., Yang, J., Ji, S., Lu, Y., Wu, P., Chen, C., 2018. Indoor air quality monitoring for the detection of SARS-CoV-2 (COVID-19) virus. Sci. Total Environ. 748, 141324.

Lednicky, J.A., Lauzardo, M., Hugh Fan, Z., et al., 2020. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients 1. medRxiv 1. https://doi.org/10.1101/2020.08.03.20167395 2020.08.01.20167395.

Lednicky, J.A., Lauzardo, M., Alam, M.M., et al., 2021. Isolation of SARS-CoV-2 from the air in a car driven by a COVID patient with mild illness. medRxiv.

Lee, J.K., Jeong, H.W., 2020. Rapid expansion of temporary, reliable airborne-infection isolation rooms with negative air machines for critical COVID-19 patients. Am. J. Infect. Control 48 (7), 822–824.

Li, X., Fan, Z.Y., Jiang, L., Wang, H.B., 2020. Aerosol and environmental surface monitoring for SARS-CoV-2 RNA in a designated hospital for severe COVID-19 patients. Epidemiol. Infect. 148 (1454), 1–5. https://doi.org/10.1017/S0950268820001570.

Liu, C., Yang, J., Ji, S., Lu, Y., Wu, P., Chen, C., 2018. Influence of natural ventilation rate on indoor PM2.5 deposition. Build. Environ. 144, 357–364.

Liu, Y., Ning, Z., Chen, Y., et al., 2020. Aerosolization of SARS-CoV-2 in two Wuhan hospitals. Nature 582 (7813), 557–560. https://doi.org/10.1038/s41586-020-2273-1.

Ma, J., Qi, X., Chen, H., et al., 2020. Coronavirus Disease 2019 patients in earlier stages exhale millions of severe acute respiratory syndrome coronavirus 2 per hour. Clin. Infect. Dis. 2020 Aug 28, ciaa1283, Brief Repo https://doi.org/10.1093/cia/ciaa1283.

Masoumbeigi, H., Ghanizadeh, G., Arfai, R.Y., et al., 2020. Investigation of hospital indoor air quality for the presence of SARS-Cov-2. J. Environ. Heal. Sci. Eng. 18 (2), 1259–1263.

Morawska, L., Cao, J., 2020. Airborne transmission of SARS-Cov-2: the world should face the reality. Environ. Int. 139 (April), 105730. https://doi.org/10.1016/j.envint.2020.105730.

Morawska, L., Milton, D.K., 2020. It is time to address airborne transmission of Coronavirus Disease 2019 (COVID-19). Clin. Infect. Dis. 2019 (X), 1–4. https://doi.org/10.1093/cid/ciaa093.

Nicar, M., Nazaroff, W., Hubbard, A., 2005. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. J. Occup. Environ. Hyg. 2 (3), 143–154. https://doi.org/10.1080/15459620590918466.

Nishiura, H., Oshitani, H., Kobayashi, T., et al., 2020. Closed environments facilitate secondary transmission of SARS-CoV-2. N. Engl. J. Med. 382 (16), 1564–1567. https://doi.org/10.1056/NEJMoa2024073.

Ong, S.W.X., Tan, V.K., Chia, P.Y., et al., 2020. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 323 (16), 1610–1612. https://doi.org/10.1001/jama.2020.3227.

Papineni, R.S., Rosenthal, F.S., 1997. The size distribution of droplets in the exhaled breath of healthy human subjects. J. Aerosol Med. 10 (2), 105–116.

Qian, H., Miao, T., Liu, L., Zheng, X., Luo, D., Li, Y., 2020. Indoor transmission of SARS-CoV-2, Indoor Air 00, 1–7.

Razzini, K., Castrica, M., Menchetti, L., et al., 2020. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. Sci. Total Environ. 742, 140540.

Rhee, C., Baker, M., Vaidya, V., et al., 2020. Incidence of nosocomial COVID-19 in patients hospitalized at a large US academic medical center. JAMA Netw. Open 3 (9), e202498.

Sanarpia, J.L., Rivera, D.N., Herrera, V.L., et al., 2020. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. Sci. Rep. 10 (1), 3–5. https://doi.org/10.1038/s41598-020-69286-3.

Siegel, J., Rhinehart, E., Jackson, M., Chiarello, L., 2007. Health Care Infection Control Practices Advisory Committee 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am. J. Infect. Control 35 (10 Sup), 505–516.

Smith, P., Anderson, A., Christoberg, G., 2006. Designing a biocontainment unit to care for patients with serious communicable diseases: a consensus statement. Biosecur. Biopre. 4, 351–365.

Smith, S.J., Eastagh, L.S., Findlay, J.S., Lever, M.S., 2020. Experimental aerosol survival of SARS-CoV-2 in artificial saliva and tissue culture media at medium and high humidity. Emerg. Microb. Infect. 9 (1), 1415–1417.

Stadnytskyi, V., Bax, C.E., Bax, A., Altmuhr, F., 2020. The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. Proc. Natl. Acad. Sci. U. S. A. 117 (22), 3–5. https://doi.org/10.1073/pnas.2006874117.

Stern, R., Koutrakis, P., Martins, M., et al., 2021. Characterization of hospital airborne SARS-CoV-2. Respir. Rev. 23, 73. https://doi.org/10.1186/s12931-021-01637-8.

Teller, R., 2006. Review of aerosol transmission of influenza a virus. Emerg. Infect. Dis. 12 (11), 1657.

Thomas, R.J., 2013. Particle size and pathogenicity in the respiratory tract. Virulence 4 (8), 847–858. https://doi.org/10.4161/viru.27172.

U.S. Department of Labor, 2020. Healthcare workers and employers. COVID-19 control and prevention. https://www.osha.gov/SLTC/covid-19/healthcare-workers.html Accessed November 19, 2020.

Van Doremalen, N., Bushmaker, T., Morris, D.H., et al., 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N. Engl. J. Med. 382 (16), 1564–1567. https://doi.org/10.1056/NEJMoa2004973.

Wan, G.H., Wu, C.L., Chen, Y.F., Huang, S.H., Wang, Y.L., Chen, C.W., 2014. Particle size concentration distribution and influences on exhaled breath particles in mechanically ventilated patients. PLoS One 9 (1), e87088.

Watanabe, T., Bartrand, T.A., Weir, M.H., Omura, T., Haas, C.N., 2010. Development of a dose-response model for SARS coronavirus. Risk Anal. 30 (7), 1129–1138. https://doi.org/10.1111/j.1539-6924.2010.01427.x.

Weissman, D.N., De Perio, M.A., Radonovich, L.J., 2020. COVID-19 and risks posed to personnel during endotracheal intubation. JAMA. 323 (20), 2027–2028.

Wells, W.F., 1934. On air-borne infection. Study II. Droplets and droplet nuclei. Am. J. Hyg. 20, 611–618.

Wölfel, R., Corman, V.M., Guggemos, W., et al., 2020. Virological assessment of hospitalized patients with COVID-19. Nature. 581 (7809), 465–469.

World Health Organization, 2014. Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. https://www.who.int/publications-i/item/infection-prevention-and-control-of-epidemic-and-pandemic-prone-acute-respiratory-infections-in-health-care Accessed November 19, 2020.

World Health Organization, 2020. Overview of public health and social measures in the context of COVID-19: interim guidance. https://apps.who.int/iris/rest/bitstreams/1278127/retrieve.

Xie, W., Fan, Y., Zhang, X., Tian, G., Si, P., 2020. A mathematical model for predicting indoor PM2.5 concentration under different ventilation methods in residential buildings. Build Serv. Eng. Res. Technol. 41 (6), 694–708.

Yang, S., Lee, G.W., Chen, C.M., Wu, C.C., Yu, K.P., 2007. The size and concentration of droplets generated by coughing in human subjects. J. Aerosol. Med. 20 (4), 484–494.

Zuo, M., Huang, Y., Ma, W., et al., 2020. Expert recommendations for tracheal intubation in critically ill patients with Novel Coronavirus Disease 2019. Chin. Med. Sci. J. 35 (2), 105–109.