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On the airborne transmission of SARS-CoV-2 and relationship with indoor conditions at a hospital

Zeynab Baboli a,b, Niloofar Neisi c, Ali Akbar Babaei d,e, Mehdi Ahmadi d,e, Armin Sorooshian f,g, Yaser Tahmasebi Birgani d,e,*, Gholamreza Goudarzi b,d,e, **

a Student Research Committee, Department of Environmental Health Engineering, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
b Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
c Clinical Sciences Research Institute, Alimentary Tract Research Center, Department of Medical Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
d Department of Environmental Health Engineering, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
e Environmental Technologies Research Center (ETRC), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
f Department of Chemical and Environmental Engineering, University of Arizona, Tucson, AZ, USA
g Department of Hydrology and Atmospheric Sciences, University of Arizona, Tucson, AZ, USA

HIGHLIGHTS

- Our findings confirm airborne transmission of SARS-CoV-2.
- Presence of an air cleaner in COVID-19 ward suppressed airway transmission of SARS-CoV-2.
- SARS-CoV-2 concentration was estimated using a mathematical model.
- Active sampling technique (vs passive sampling) identified more SARS-CoV-2 positive cases.
- Low temperature and low humidity coincided with the presence of SARS-CoV-2.

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ABSTRACT

The limited knowledge about the mechanism of SARS-CoV-2 transmission is a current challenge on a global scale. Among possible transmission routes, air transfer of the virus is thought to be prominent. To investigate this further, measurements were conducted at Razi hospital in Ahvaz, Iran, which was selected to treat COVID-19 severe cases in the Khuzestan province. Passive and active sampling methods were employed and compared with regard to their efficiency for collection of airborne SARS-COV-2 virus particles. Fifty one indoor air samples were collected in two areas, with distances of less than or equal to 1 m (patient room) and more than 3 m away (hallway and nurse station) from patient beds. A simulation method was used to obtain the virus load released by a regularly breathing or coughing individual including a range of microdroplet emissions. Using real-time reverse transcription polymerase chain reaction (RT-PCR), 11.76% (N = 6) of all indoor air samples (N = 51) collected in the COVID-19 ward tested positive for SARS-CoV-2 virus, including 4 cases in patient rooms and 2 cases in the hallway. Also, 5 of the 6 positive cases were confirmed using active sampling methods with only 1 based on passive sampling. The results support airborne transmission of SARS-CoV-2 bioaerosols in indoor air.

* Corresponding author. Environmental Technologies Research Center (ETRC), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
** Corresponding author. Air Pollution and Respiratory Diseases Research Center (APRD), Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
E-mail addresses: tahmasebi-y@ajums.ac.ir (Y.T. Birgani), rezagoudarzi1350@gmail.com, ghgoodarzi@ajums.ac.ir (G. Goudarzi).
1. Introduction

In December 2019, a new coronavirus was identified as causing pneumonia cases in Wuhan City, Hubei Province, China. In February 2020, the World Health Organization (WHO) called the disease COVID-19, also known as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Subsequently on March 2020, the WHO declared a global pandemic. The COVID-19 disease spread rapidly away from China to other regions with an increasing number of cases reported in other countries around the world. On 19 February 2020, the first SARS-CoV-2 confirmed case was reported in Iran. From 19 February 2020 to 8 June 2021 in Iran, there have been 2,971,270 confirmed cases of COVID-19 with 81,183 deaths reported to the WHO (https://covid19.who.int/region/emro/country/ir). The WHO reported that the main transmission route of novel coronavirus is person-to-person transmission (WHO, 2020b). Therefore, the specific transmission routes and dynamics of SARS-CoV-2 virus are still under investigation.

Viruses are a common cause of infectious disease and they can be easily transmitted in crowded indoor spaces with poor air ventilation including in hospitals and public places. Some researchers have reviewed the viral disease transmission routes, which lead to respiratory issues (La Rosa et al., 2013; Mitchell et al., 2018). The main transmission routes of respiratory viruses are direct contact, respiratory droplets, and airborne transmission. Spread of the virus via direct contact and respiratory droplets may be controlled by infection control measures, including hand washing and wearing face masks, but the control of airborne transmission is difficult, as viruses are likely to attach to suspended particles and they can stay in the air for prolonged periods of time (Mitchell et al., 2018, Spena et al., 2020). Also, they can be released as respiratory droplets and deposit on surfaces when an infected individual coughs, sneezes, or talks. Infection can occur if people touch the contaminated surface and then touch their eyes, nose, or mouth and can also infect other people if there is direct contact and via inhalation (Razzini et al., 2020; Zhang et al., 2020; Ma et al., 2020; Sohrabi et al., 2020; Wang et al., 2020; WHO, 2020a; HCP, 2019; WHO, 2020a).

SARS-CoV-2 transmission routes are similar to the transmission of influenza, SARS-CoV-1, and MERS-CoV, occurring mainly through respiratory droplets and airborne aerosols (Van Doremalen et al., 2020; Morawska and Cao, 2020; Mitchell et al., 2018). However, limited knowledge about the mechanism of transmission has been a challenge for public health decision and policy makers, especially early on during the COVID-19 pandemic. Among the transmission routes introduced, air transfer of the virus is widely accepted (Kumar and Morawska, 2019; Morawska and Cao, 2020; Morawska et al., 2020; Van Doremalen et al., 2020; Guan et al., 2020; Kenarkooi et al., 2020). However, there have been studies unable to detect the virus in air samples (Ong et al., 2020; Faridi et al., 2020; Masoumbeigi et al., 2020; Cheng et al., 2020), while others have been able to detect the virus in some air samples (Razzini et al., 2020; Kenarkooi et al., 2020; Chia et al., 2020; Jiang et al., 2020; Liu et al., 2020; Lednicky et al., 2020a, 2020b; Ding et al., 2020; Santarpia et al., 2020).

The main purpose of policies and restrictions imposed by different countries during the COVID-19 (e.g., travel bans, screening and testing of travelers, quarantine, closure of schools and organizations) is to reduce contact between infected and susceptible individuals to reduce spreading. A more accurate understanding of the routes of transmission can help find more effective ways to control this disease (Wei and Li, 2016; Kumar and Morawska, 2019; Morawska and Cao, 2020). Due to the contradictions and lack of sufficient understanding of the nature of this virus, the airborne route of transmission needs to be examined more closely. Indoor and outdoor air conditions are an important factor for virus viability and also an environmental risk factor in the spread of COVID-19. Ambient air quality can also impact indoor pollution concentrations (Moller, 2020). Therefore, there is a need to examine transmission rates of viral diseases, the virus transmission routes, and the relationship among indoor air quality factors, the survival of the virus, and presence/absence of viruses in aerosol particles and on surfaces.

Shortly after the COVID-19 outbreak, the Razi hospital in the middle of Ahvaz city (Iran) was selected by health care decision makers and authorities as a focal point of treating patients with the COVID-19 disease within the province of Khuzestan. Although many months have passed since the outbreak of coronavirus in Iran, the Khuzestan province, particularly the city of Ahvaz, is still in a very vulnerable situation. The objective of the present study was to detect SARS-CoV-2 RNA at indoor air of Razi hospital using RT-PCR, and to study the effect of indoor air conditions on its transmission.

2. Materials and methods

2.1. Study design and site selection

This study was conducted between 15 July and 2 August 2020 in a COVID-19 patient ward, comprised of separate infectious and ICU wards, of Razi hospital in Ahvaz, Iran (Fig. 1). For the study period, the infectious ward contained 22 beds and 22 patients, while the ICU ward had 4 beds and 3 patients who tested positive for SARS-CoV-2.

2.2. Description of air sampling procedure

During this study, two types of aerosol sampling methods were employed and we compared the efficiency of the two methods for collection of SARS-CoV-2 virus in air. The first attempt was passive sampling, in which standard Petri dishes (settle plates) with 8 mm diameter containing 5 mL of liquid media were placed openly at sampling points (Fig. 1) for 30 min (Napoli, 2012). The second approach was active sampling and conducted three ways for 30 min per sample: glass impinger (AGI) that was connected to SKC universal air sampling pumps at a flow rate of 4 L per min; the SKC universal air sampling pumps with 37 mm and 0.3 μm pore size polytetrafluoroethylene membrane filters (PTFE) at a flow rate of 4 L per min; the Petri dishes with 8 mm diameter containing 5 mL of liquid media were placed in the bioاستغلاط of a Quick Take 30 kit set sampling at a flow rate of 4 L per min. Both pumps (SKC and Quick Take 30) were calibrated using an electronic calibrator (Bios Defender 510 H, USA) (Santarpia et al., 2020; Napoli et al., 2012; Booth et al., 2005; Coleman et al., 2018).

Each sampler collection vessel was pre-filled with 5 mL of Dulbecco’s Modified Eagle’s Medium liquid (DMEM) or 5 mL of normal saline liquid (Kenarkooi et al., 2020; Faridi et al., 2020). The collected liquid samples were immediately transfer to 15 mL sterile falcons under sterile conditions and stored at 4 °C. They were then transported to a clinical virology laboratory and frozen at −70 °C before SARS-CoV-2 RNA extraction. Prior to sampling, all experimental setups such as glass sampling devices were autoclaved and disinfected using an ethanol 70% solution (Kenarkooi et al., 2020; Faridi et al., 2020; Booth et al., 2005; Verreault et al., 2008; Chen et al., 2004).

In COVID-19 wards, the air conditioning systems did not work effectively, but there are several split coolers in corridors and rooms to cool down the temperature. The temperature (°C) and relative humidity (%) were measured using AQ110 and HD 110 portable weather stations (KIMO), respectively.

Locations (sampling points) 1–3 as well as locations 4–7 are shown in...
Fig. 1. Air samplers including impinger-pump coupling, biostage samplers (Quick take 30), filters, portable aerosol monitors, and temperature-humidity sensors were placed at distances more than 3 m from patients for 22 samples collected at locations 1–3. This equipment was also installed at distances equal or less than 1 m from patients for 29 samples collected at locations 4–7. Several active and passive samples were taken at the same sampling point.

All samplers operated at a height of 1.5–1.8 m from the floor to represent the breathing zone of patients while patients were present in the room (Nguyen et al., 2017). The distance between patients’ beds was 1 m without any physical separator. It is noteworthy that patients did not wear face masks and they were not connected to ventilators, but they had fever, cough, and body aches during air sample collection. The sampling team and all medicine staff members wore a disposable plastic apron equipped with respiratorators during sampling activities. This means that the SARS-CoV-2 bioaerosols are unlikely to have been generated by anyone other than the patients (Mitchell et al., 2018; Lednicky et al., 2020a).

2.3. Real-time PCR

The collected air samples were transferred to a virology lab in cold chain and were kept at −70 °C. Total RNAs were extracted using an RNA Extraction Kit (Sinaclon Co, Tehran, IRAN). The purity of extracted RNAs was determined by Nanodrop (ThermoScientific, USA). One-step reverse transcriptase real-time PCR was performed for RNA-dependent RNA polymerase (RdRp) and N SARS-CoV-2 genes with specific primers and probes (FAM and Texas red) with the use of a One Step Novel Coronavirus (2019-NCOV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing, SANSURE Biotech, China). The internal control primer and probe (CYS) were evaluated. Thermal cycling conditions were as follows: 50 °C for 30 min, 95 °C for 1 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s. The RT-PCR cycle threshold (CT) values ≤ 40 and a sigmoidal curve were considered as positive results for both genes. The sampling and extraction were repeated for samples in which the Ct of the Internal Control gene was not determined.

2.4. Estimating viral load concentration

Viral load concentration was simulated for the condition of SARS-CoV-2 RNA copies released by a regularly breathing or coughing individual. The viral emission factor was fed into a well-mixed 1-compartment model to simulate the situation in a closed room with different ventilation air exchange rates (AER) using Equation (1), the parameters within which are defined in Table 1 (Riediker and Tsai, 2020).

\[
V_b \cdot \frac{dC}{dt} = C_{pm} \cdot RR \cdot V_t - V_b \cdot AER \cdot C(t) - \frac{\ln(2)}{t_2} \cdot V_b \cdot C(t)
\]  

We simulated a range of viral emission rates for an individual including low, typical, and high emissions of microdroplets as 1000, 10^6, and 1.3 × 10^11 copies mL\(^{-1}\), respectively.

(Riediker and Tsai, 2020).

2.5. Data analysis

Indoor air quality parameters of Razi hospital were analyzed based on simple statistical methods and multivariate analysis such as principal component analysis (PCA) and self-organizing maps (SOMs). Multivariate analysis was employed in order to understand and also prove statistically significant relationships between PCR results with meteorological parameters as well as other parameters. PCA and SOMs are commonly used for analysis of complex data structures, non-linear problems, classification, prediction, modeling, and data mining (Rad et al., 2019; Gulson et al., 2007; Olikowska et al., 2014; Kwon et al., 2015; Rösch et al., 2014). Principal component analysis (PCA) is used as a way to reduce the dimensionality of this study’s dataset. In PCA, the main variables are orthogonally converted to obtain a new set of variables that includes a principal component (PC), and a symmetrical change is used to adjust an arrangement of perceptions of the corresponding potential variables into an arrangement of directly uncorrelated factors called essential segments. The method makes it possible to reduce the dimension of a large dataset containing a large number of variables, while simplifying the interpretation of the results. Results are

Table 1
Parameters relevant to Equation (1) required to estimate viral load concentration in a closed space.

| Parameters | Definition                                      | Unit & value |
|------------|-------------------------------------------------|--------------|
| C          | emission rate of viral concentration            | Copies per m\(^2\) |
| C\(_{pm}\) | viral load in the PM\(_{10}\) size range        | Copies per particles m\(^2\) |
| V\(_{t}\)  | room volume                                     | 500 mL per breath |
| RR         | respiratory rate                                 | 15 breaths per minute |
| AER        | air exchange rate                               | times per hour  |
| t\(_{0}\)  | virus’ half-life                                 | 1.1 h |

Fig. 1. Schematic diagram of sampling area and air-sampler locations in the infectious and ICU wards of Razi hospital.
also presented in the form of biplots, where variables are represented as vectors (bars) providing information three ways (Linting et al., 2007; Rossiter, 2014; Rad et al., 2019; Kwon et al., 2015): (i) orientation (direction) of vector in contrast to the principal component space whereby a vector more parallel to a PC axis, the more it contributes to that PC; (ii) angles between vectors representing different variables, whereby small angles represent strong positive correlations and opposite angles represent high negative correlations; and (iii) length, whereby longer vectors represent higher variability represented by two displayed PCs.

Kohonen maps (i.e., SOMs) are constructed based on a neural network and provide a two-dimensional map revealing potential data similarities. We used the SOM Toolbox in Matlab 6.0, which has a SOM for each parameter and a U-matrix (unified distance matrix), which signifies the Euclidean distance between adjacent neurons and shows the level of “similarity” between neurons. Similarities between SOMs provide an indication of interrelationships between the different variables (Kwon et al., 2015; Zuska et al., 2019; Katsifarakis and Karatzas, 2017).

In our study, the independent principal components are 14 parameters including: temperature (T, °C); relative humidity (RH, %); air velocity (AV, m/s); PM$_{10}$ (particulate matter with diameter ≤ 10 μm); PM$_{2.5}$ (particulate matter with diameter ≤ 2.5 μm); PM$_{1}$ (particulate matter with diameter ≤ 1 μm); distance between samplers and patients (Dis, m); patient population by the sampling point (PPS); day of confirmed illness of patients (DCI); presence or absence of air cleaner (AC); area of sampling point (A, m$^2$); type of sampler (S); type of transfer liquid (TL); type of ventilation system (VS); and the dependent variable is either the presence or absence of SARS-CoV-2 in the indoor air samples.

3. Results

In the present study, passive and active air sampling methods were used for indoor air sampling in the COVID-19 wards of Razi hospital. The number of air samples was 51, of which, 29 were collected in patient rooms located (i.e., less than or equal to 1 m away from patient bed) and 22 were collected in the hallway (i.e., more than 3 m away from patient beds). Tables 2 and 3 summarize conditions of the indoor air sampling methods and results of RT-PCR for SARS-CoV-2 virus.

Using real-time reverse transcription polymerase chain reaction (RT-PCR), 11.76% (N = 6) of all indoor air samples (N = 51) collected in the COVID-19 ward tested positive for SARS-CoV-2 virus. This included 4 positive cases (7.84% of all samples) in patient rooms and 2 positive cases (3.92% of all samples) in the hallway. As shown in Tables 2 and 3, active sampling methods accounted for 5 of the 6 positive confirmed cases while 1 case was confirmed using passive sampling.

The ventilation and air cleaner flow rate at different sampling points are presented in Table 4. For comparison with results of the present study, standard values from the Ninomura and Bartley (2001) study were added in Table 4. At sampling points where air cleaners were available, the device flow rate was added to the ventilation rate and the virus concentration was calculated and presented using software provided by Redlaker and Tsal (2020). To compare with values of standard AER in the hospital environment, AER values at sampling points 1, 3, and 5 were comparable with the standard, while the AER values for the other points were lower than the AER standard values. At points 2 and 6 where the air samples were positive for SARS-CoV-2, the ventilation rates were low and there was no air cleaner device present; consequently, an inverse relationship is observed between positive samples for SARS-CoV-2 and the presence of an air cleaner, as shown by PCA and SOM analysis. Also, estimates of virus concentration are consistent with the results of virus detection in air samples in that there was a positive relationship.

Multivariate analysis (PCA and SOM) was applied to the dataset and Fig. 2–3 summarize results. In this study, we used PCA to investigate statistically significant relationships between the presence/absence of airborne SARS-CoV-2 and multiple indoor air factors. The main variables are orthogonally transformed to obtain a new set of variables that

| Sampling point | Samples No | Type of sampler | Transfer liquid | SARS-CoV-2 in air samples | Area (m²) | Air velocity (m/s) | Temperature (°C) | Humidity (RH %) | Air cleaner system$^a$ | PM$_{10}$ (μg/m$^3$) | PM$_{2.5}$ (μg/m$^3$) | PM$_{1}$ (μg/m$^3$) |
|---------------|------------|-----------------|----------------|--------------------------|-----------|-------------------|-----------------|----------------|-------------------------|----------------|----------------|----------------|
| 1             | 1,2        | Active (Impinger) | DMEM, NS       | Negative                  | 60        | 0                 | 25.5            | 61.2           | 1                       | 39             | 35             | 32             |
|               | 3,4        | Active (Quick take) | DMEM, NS       | Negative                  | 60        | 0                 | 24.3            | 42             | 0                       | 72             | 65             | 58             |
|               | 5,6        | Passive         | DMEM, NS       | Negative                  | 60        | 0                 | 24.3            | 42             | 0                       | 72             | 65             | 58             |
|               | 7          | Active (SKC, Filter) | DMEM, NS       | Negative                  | 60        | 0                 | 24.3            | 42             | 0                       | 72             | 65             | 58             |
| 2             | 8,9        | Passive         | DMEM, NS       | Negative                  | 60        | 0                 | 24.3            | 42             | 0                       | 72             | 65             | 58             |
|               | 10,11      | Active (Quick take) | DMEM, NS       | Negative                  | 60        | 0                 | 24.3            | 42             | 0                       | 72             | 65             | 58             |
|               | 12         | Active (Impinger) | DMEM, NS       | Positive                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 13         | Active (Impinger) | NS             | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 14         | Active (SKC, Filter) | DMEM, NS       | Positive                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 15         | Active (SKC, Filter) | DMEM, NS       | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
| 3             | 16,17      | Active (Impinger) | DMEM, NS       | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 18,19      | Active (Quick take) | DMEM, NS       | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 20,21      | Passive         | DMEM, NS       | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |
|               | 22         | Active (SKC, Filter) | DMEM, NS       | Negative                  | 12        | 0.2               | 24.9            | 54.5           | 1                       | 49             | 33             | 30             |

$^a$ 0 and 1 = Absence (0) and presence (1) of air cleaner system.
includes a principal component (PC). In our study, 51 samples, with each having the same 15 parameters recorded, were collected from the COVID-19 ward of Razi hospital. According to the PCA results, Fig. 2a shows that among the 15 parameters examined, two principle components (PCs) had the greatest impact on PCR results (PC1 and PC2). Fig. 2b shows the two-dimensional biplot of PC1 versus PC2, and the role of each of the indoor air parameters in affecting the PCR results. The observations in the plot show the original variables as vectors (bars). Their specifications are [0, 0] [0, 0] at the origin and extend to coordinates given by the loading vector [0.4, 0.4] [0.4, 0.4]. In the biplot, each point represents sampling points’ data, and each line (bar) represents one of the study variables. The first PC (Fig. 2a and b horizontal axis) explains 38.45% of the total variance and is positively affected (right) by SARS-

### Table 3
Detection of SARS-CoV-2 in the patient room COVID-19 ward (equal or less than 1 m away from the patient beds), sampling methods, and indoor air parameters.

| Sampling point | Samples NO | Type of sampler | Transfer liquid | SARS-CoV-2 in air samples | Area (m²) | Air velocity (m/s) | Temperature (°C) | Humidity (RH%) | Air cleaner system* | PM₁₀ (µg/m³) | PM₂,₅ (µg/m³) | PM₁ (µg/m³) |
|----------------|------------|-----------------|----------------|--------------------------|-----------|-------------------|-----------------|----------------|-------------------|--------------|---------------|-------------|
| 4              | 23,24      | Active (Impinger) | DMEM, NS       | negative                | 35        | 0.3               | 24.4            | 61.2           | 1                 | 46           | 42            | 39          |
|                | 25,26      | Active (Quick take) | DMEM, NS       |                          |           |                   |                 |                |                   |              |               |             |
|                | 27,28      | Passive          | DMEM, NS       |                          |           |                   |                 |                |                   |              |               |             |
|                | 29         | Active (SKC, Filter) | DMEM            |                          |           |                   |                 |                |                   |              |               |             |
| 5              | 30,31      | Active (Impinger) | DMEM, NS       | negative                | 40        | 0.16              | 24.7            | 51.3           | 1                 | 61           | 50            | 47          |
|                | 32,33      | Active (Quick take) | DMEM, NS       |                          |           |                   |                 |                |                   |              |               |             |
|                | 34,35      | Passive          | DMEM, NS       |                          |           |                   |                 |                |                   |              |               |             |
|                | 36         | Active (SKC, Filter) | DMEM            |                          |           |                   |                 |                |                   |              |               |             |
| 6              | 37         | Passive          | DMEM, NS       | negative Positive       | 12        | 0.25              | 23.9            | 45             | 0                 | 80           | 73            | 69          |
|                | 38         | Active (Quick take) | NS             |                          |           |                   |                 |                |                   |              |               |             |
|                | 39         | Active (Impinger) | DMEM            | Positive                |           |                   |                 |                |                   |              |               |             |
| 7              | 40         | Active (Quick take) | NS             | Negative                |           |                   |                 |                |                   |              |               |             |
|                | 41         | Active (Impinger) | DMEM            | Positive                |           |                   |                 |                |                   |              |               |             |
| 8              | 42         | Active (Impinger) | NM              | Negative                |           |                   |                 |                |                   |              |               |             |
| 9              | 43         | Active (SKC, Filter) | DMEM            | Positive                |           |                   |                 |                |                   |              |               |             |
| 10             | 44         | Active (SKC, Filter) | DMEM            | Negative                |           |                   |                 |                |                   |              |               |             |
| 11             | 45,46      | Active (Impinger) | DMEM, NS       | positive                | 20        | 0.33              | 26.3            | 50.6           | 0                 | 17           | 10            | 8           |
|                | 47,48      | Active (Quick take) | NS             |                    |           |                   |                 |                |                   |              |               |             |
|                | 49,50      | Passive          | DMEM, NS       |                          |           |                   |                 |                |                   |              |               |             |
|                | 51         | Active (SKC, Filter) | DMEM            |                          |           |                   |                 |                |                   |              |               |             |

### Table 4
Summary of SARS-CoV-2 concentrations at various sampling points of the COVID-19 ward.

| Sampling point | Location | Ward  | Ventilation system a | Volume room (m²) | Ventilation flow rate (m³/s) | Air cleaner flow rate (m³/s) | Present study AER (time/h) | Standard AER b (time/h) | Estimated Viral load c (copies/m³) | Positive air samples (number) |
|----------------|----------|-------|----------------------|------------------|-------------------------------|-------------------------------|-----------------------------|---------------------------|-----------------------------------|-------------------------------|
| 1              | Hallway  | Infectious | C&F                  | 180              | 0.5                           | 0                            | 10                          | 2                         | 0.22                             | 0                             |
| 2              | Hallway  | Infectious | C&F                  | 180              | 0                            | 0                            | 0                           | 2                         | 2.53                             | 2                             |
| 3              | Nurse    | Infectious | C&F                  | 36               | 0.05                          | 0.2                          | 23                          | 2                         | 0.24                             | 0                             |
| 4              | Station  | Infectious | C&F                  | 105              | 0.135                         | 0.83                         | 4.6                         | 6                         | 1.43                             | 0                             |
| 5              | Patient  | Infectious | C&F                  | 120              | 0.072                         | 3.75                         | 6.93                        | 12                        | 0.86                             | 4                             |
| 6              | Room     | Infectious | NP                   | 60               | 0.115                         | 6.93                         | 12                          | 0                         | 0.18                             | 0                             |
| 7              | Patient  | ICU     | NP                   | 60               | 0.115                         | 6.93                         | 12                          | 0                         | 0.18                             | 0                             |

a C&F= Cooler and fan; NP= Negative pressure.
b Standard AER (Ninomura and Bartley, 2001).
c The estimated of SARS-CoV-2 concentration in a closed room for different air exchange rates (Riediker and Tsai, 2020).
The presence of SARS-CoV-2 and multiple indoor environmental parameters at sampling points are given in Table 5. The variable coefficients in PC1 and PC2 in Table 5 confirm results of Fig. 2b, and the bold coefficients are the same as the effective coefficients for each component. The positive and negative coefficients indicate a direct or reverse effect, respectively, on each component.

The red circles in Fig. 2b represent inter-relationships between PM (PM$_{10}$, PM$_{2.5}$, and PM$_1$) variations, revealing they are closely related to each other. The green circle in Fig. 2b shows that the presence of SARS-CoV-2 bioaerosols in the collected samples (positive PCR) was negatively correlated with temperature and relative humidity. In terms of vector properties (orientation, angle, and length), PM$_{10}$, PM$_{2.5}$, and PM$_1$ are more parallel to SARS-CoV-2 indicating a high contribution to SARS-CoV-2 and virus concentration. In other words, the higher the PM level, the higher the concentration of SARS-CoV-2. No angle was observed between PM$_1$, PM$_{2.5}$, and PM$_{10}$ indicating highly positive correlation between these size fractions of particles inside the ward. A negligible and positive (right) angle was observed between these three fraction sizes and SARS-CoV-2 supporting a positive correlation between PM and virus load.

We next examine SOMs to get a better understanding of the relationship between the presence/absence of indoor air SARS-CoV-2 and indoor air factors in the hospital environment. The interrelationships between data are presented as areas of similar color and shape in the SOM graphs, identified with the aid of the U-matrix graph. In the U-matrix, the blue and red colors correspond to low and high values, respectively, of the corresponding variables. The SARS-CoV-2 parameter in Fig. 3 as well as the U-matrix indicates that there are two main areas of the specific profile, each one related to a different part of the SARS-CoV-2 behavior: the upper area corresponds to low SARS-CoV-2 (marked with blue circles in the SARS-CoV-2 SOM and other SOMs) and similar with medium to low PMs, AC, and VS and not similar with RH%, T, DCI, VS, S, and TLS. The bottom area corresponds to high SARS-CoV-2 (marked with red circles in the SARS-CoV-2 SOM and other SOMs) and similar with medium to high PMs and AC, and not similar to RH%, T, DCI, S, and TLS. The presence of SARS-CoV-2 virus can be attributed to high PMs and the lack of AC and also low RH%, T, DCI, S, and TLS. Fig. 3 shows that the color patterns of PM$_{10}$, PM$_{2.5}$, and PM$_1$ are similar suggesting that variations are closely related to each other; more specifically, the color pattern of SARS-CoV-2 bioaerosols in the collected samples (black box) is similar to color pattern of PM$_{10}$, PM$_{2.5}$, and PM$_1$ mass concentrations (red box). The color patterns of relative humidity and temperature variations (blue box) are related and in the opposite direction of SARS-CoV-2 bioaerosols in the collected samples; thus, the presence of SARS-CoV-2 in the collected samples (via positive PCR) was negatively correlated with temperature and relative humidity. In terms of vector properties (orientation, angle, and length), PM$_{10}$, PM$_{2.5}$, and PM$_1$ are more parallel to SARS-CoV-2 indicating a high contribution to SARS-CoV-2 and virus concentration. In other words, the higher the PM level, the higher the concentration of SARS-CoV-2. No angle was observed between PM$_1$, PM$_{2.5}$, and PM$_{10}$ indicating highly positive correlation between these size fractions of particles inside the ward. A negligible and positive (right) angle was observed between these three fraction sizes and SARS-CoV-2 supporting a positive correlation between PM and virus load.

Table 5

| Variable | PC1      | PC2      |
|----------|----------|----------|
| S        | -0.01018 | 0.00955  |
| VS       | -0.34555 | -0.19973 |
| TL       | -0.02451 | 0.034125 |
| Dis(m)   | 0.113877 | 0.445363 |
| A(m$^3$) | 0.111577 | 0.36556  |
| AV (m/s) | -0.21806 | -0.39988 |
| T(◦C)    | -0.3717  | 0.199335 |
| RH (%)   | -0.19054 | 0.305415 |
| AC       | 0.050405 | -0.35091 |
| PPs      | -0.06325 | -0.22226 |
| DCI      | -0.34407 | -0.24732 |
| PM$_{10}$| 0.398311 | -0.14159 |
| PM$_{2.5}$| 0.398328 | -0.14344 |
| PM$_1$   | 0.394829 | -0.15406 |
| SARS-CoV-2| 0.185272 | -0.23721 |

Fig. 2. PCA analysis of the presence of SARS-CoV-2 in the COVID-19 ward and all indoor parameters: a) Pareto diagram, and b) PCA Biplot of the first two principal components (PC1 versus PC2) and visual correlation of SARS-CoV-2 with the most important factors affecting indoor air quality. Scores for variables are plotted versus the bottom and left axes.
PCR) was negatively correlated with temperature and relative humidity. Based on PCA analysis, there was no specific relationship between the sampling methods (S), liquid transfer (TL), and day of confirmed illness of patients (DCI) variables with SARS-CoV-2; in contrast, the SOMs show an inverse relationship between these parameters. Color patterns of S, TL, and DCI variations (green boxes) are in the opposite direction of the color pattern of SARS-CoV-2 bioaerosols in the collected samples. Therefore, the active sampling methods are more effective than passive methods for detecting SARS-CoV-2 in the collected samples. Also, the DMEM liquid transfer is better than NS for sampling the airborne virus.

4. Discussion

Our results show that the SARS-CoV-2 virus was detected in 13.8% and 9.1% of air samples at a distance of less than or equal 1 and more than 3 m away from the patient, respectively. Recent studies detected SARS-CoV-2 in indoor air samples of COVID-19 wards. For instance, a high positive rate of SARS-CoV-2 was reported by Razzini et al. (100%) (Razzini et al., 2020), Lednicky et al. (50% and 66.7%) (Lednicky et al., 2020b), Santarpia et al. (63.2% in patient room and 66.7% in hallway) (Santarpia et al., 2020), Chia et al. (66.7%) (Chia et al., 2020), and Liu et al. (73.9%) (Liu et al., 2020). In contrast, a low positive rate of SARS-CoV-2 was reported by Kenarkohi et al. (33%) (Kenarkohi et al., 2020), Guo et al. (23.5%) (Guo et al., 2020), and Jiang et al. (3.57%) (Jiang et al., 2020). Santarpia et al. (2020) suggested that viral bioaerosols are produced by COVID-19 patients. Viral RNA positive air samples indicate virus-containing particles are derived from infected patients and transported from their rooms externally to sample locations exceeding 2 m of separation (Santarpia et al., 2020). Another study showed that SARS-CoV-2-containing particles were widely distributed in indoor air from COVID-19 wards, exceeding distances of 4 m (Guo et al., 2020). While Ong et al. and WHO reported that SARS-CoV-2 is not airborne and the air transmission route has no role in the transmission of SARS-CoV-2 (Ong et al., 2020; WHO, 2020c), our results indicate that the SARS-CoV-2 virus is airborne. Virus detection at distances of more than 2 m from the virus source is classified as airborne transmission (CDC, 2020), so our results and those of others provide evidence of this transmission route.

Due to the presence of SARS-CoV-2 in hallway samples and the spread of the virus at more than 3 m away from patient beds, air conditioning and ventilation alone in the COVID-19 ward was insufficient for removal of SARS-CoV-2 from the air (Brown et al., 2015). These findings are important for decision-makers to execute actions for medical staff, visitors, and patients to prevent airborne virus transmission.

Detection of SARS-CoV-2 is challenging and here we used two sampling methods because some researchers did not detect SARS-CoV-2 virus aerosols in indoor air of hospital wards using active methods such as liquid impinge biosamplers (Faridi et al., 2020; Masoumbeigi et al., 2020), gelatin filters (Brown et al., 2015), and filter cassettes (Ong et al., 2020). SARS-CoV-2 was detected here using both active and passive sampling methods, although the active method revealed a higher rate of detection. As shown in Tables 2 and 3, the percent of virus-positive samples for active and passive sampling was 13.5% (5) and 7.14% (1), respectively. Based on Fig. 3, the highest correlations with positive SARS-CoV-2 air samples were obtained for the active air sampling and liquid transfer methods; consequently, the active sampling methods are more effective than the passive methods for detecting SARS-CoV-2 in the collected samples. Also, DMEM liquid transfer is better than NS for sampling, retaining, and detecting the airborne virus. Differences in the air sampling methods could have an influence on...
sampling efficiency and detection of virus (Coleman et al., 2018; Verreault et al., 2008; Dybwad et al., 2014). Wu et al. and Dybwad et al. indicated that they could not detect SARS-CoV-2 in air samples owing to low signal and recommended that higher air volumes should be collected using air samplers for easier detection of low virus aerosol concentrations. Understanding the capabilities of available air samplers that can characterize bioaerosol properties such as concentration and size, specifically for field work (including indoor areas), will help fill the knowledge gap about airborne transmission (Wu et al., 2020; Dybwad et al., 2014).

We observed that the presence of virus in air samples exhibits a strong inverse relationship with the day of confirmed illness of patient variations; in the other words, the positive SARS-CoV-2 air samples increased in the early days of DGI of patients. It was apparent that the spread of the virus decreased in the final days of the illness. Chia et al. showed that air samples for detecting SARS-CoV-2 on days 5 and 9 of the illness for COVID-19 patients were positive and negative, respectively, indicating the highest plausible presence of SARS-CoV-2 in the air samples during the first week of illness (Chia et al., 2020). Our multivariate analysis showed that among the 15 parameters studied, the highest correlations with positive SARS-CoV-2 air samples were obtained for indoor air parameters including temperature, relative humidity, PM concentration, and the presence of air cleaner devices. We found the presence of virus in air samples has an inverse relationship with T and RH variations, and a strong direct relationship with PM concentration and the absence of air cleaner devices. More data is needed to investigate the role of other studied parameters on the presence of virus in the air samples.

Previous investigation in Singapore revealed that positive indoor air samples for respiratory viruses such as adenovirus, RSV-A, and influenza A virus were 64%, 29%, and 7%, respectively using NIOSH sampler, PCR, and RT-PCR. They found that bioaerosols at higher RH levels (>50%) deposit more quickly; therefore, high RH levels (63%) could explain the low recovery of virus-positive aerosol samples and is not theoretically conducive to heavy airborne transmission of influenza viruses. The low load of viral bioaerosol may lead to complexity in detecting suspended viruses in air, indicating a decrease in the sensitivity of virus sampling (Coleman et al., 2018). Similar to our results, previous studies found that the risk of transmission or the persistence of other viruses such as influenza virus were reduced at warmer temperatures (30 °C) (Casanova et al., 2010). Also in indoor environments with RH lower than 40%, droplets from a cough or a sneeze lose their moisture quickly and these dry aerosols stay in the air for longer periods and viral particles remain infectious much longer (Lowen and Steel, 2014). Particulate matter with diameter equal or less than 2.5 μm are known to carry microbiomes, so they can remain suspended in the air, while large droplets fall to the ground (Feng et al., 2016; Qin et al., 2020). A positive relationship between the influenza-like illnesses and levels of PM2.5 was observed (Feng et al., 2016). Therefore, these investigations suggest that ways to minimize the risk of infection include having high temperature and relative humidity and low mass concentration of particles.

Recent studies discussed the association between morbidity and mortality of COVID-19 cases with outdoor meteorological parameters (Wangb et al., 2020; Croft et al., 2019; Setti et al., 2020), but our study is the first laboratory study to investigate the relationship between the presence of the virus in indoor air samples with indoor air conditions. Our study has the same limitations as the previous studies, with the presence of one or more COVID-19 patients who spread the virus in various ways such as breathing, coughing, and speaking. The estimated virus concentration depended on aerosol formation processes and ventilation rates. The range of estimated viral load by simulated breathing and coughing individuals were 0.0049–637000 copies m⁻³ and 277 to 3603000000 copies m⁻³, respectively. The estimate of viral load suggested that the virus concentration increased in the air of small and poorly ventilated patient rooms particularly with high emission during early days of illness (Riediker and Tsai, 2020). Similar to the Riediker and Tsai study, we observed that virus concentrations were higher in areas with lower ventilation rates or lack of an air cleaner, so this indicates the important role of ventilation in the viral load of indoor environment.

Based on the WHO and U.S. Centers for Disease Control and prevention (U.S.CDC), three strategies to reduce indoor airborne contaminants include source control, ventilation, and air cleaning systems. Our results show that it is very important to optimize the relative humidity, PM mass concentration, and temperature of indoor environments, along with improved ventilation systems and air cleaners to reduce the stability and spread of airborne SARS-CoV-2 (Razzini et al., 2020; Ahlawat et al., 2020; Kumar and Morawska, 2019; Morawska and Cao, 2020).

Our study has some limitations. More air samples are needed in different conditions and places, so that we can more accurately investigate the effect of environmental parameters on the transmission of SARS-CoV-2. It is also recommended that, in addition to detection of the virus in air samples, the viral load and viral culture be examined to determine the infectious dose of airborne SARS-CoV-2.

5. Conclusion

Our findings confirm that the SARS-CoV-2 is airborne and that air transmission is a major route of transfer between people in indoor places. According to our findings, the most important conditions affecting, or at least coincident, with the presence of the virus in indoor air of a COVID-19 ward are low temperature, low humidity, high PM levels, and absence of an air cleaner. Thus, those parameters can be optimized in indoor places to reduce transmission. Our results emphasize that sufficiently high ventilation rates and the presence of air cleaners are ways to improve indoor air quality. Other basic control measures are encouraged such as maintaining physical distance and wearing personal protective
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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