Indoor, outdoor and ICU Environment monitoring of COVID-19

Nathalia Villa Santos

Faculdade de Saúde Publica da USP

Dunia Waked

University of Sao Paulo School of Medicine

Zofia Wicik

University of Warsaw

Michele Soares Gomes-Gouvêa

University of São Paulo

João Renato Rebello Pinho

University of São Paulo

Anna Sara Shefferman Levin

University of São Paulo

Yeh-Li Ho

University of São Paulo

Mariana Matera Veras (✉ verasine@usp.br)

University of Sao Paulo School of Medicine

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Abstract

The disease caused by the SARS-CoV-2 virus, which originated in Wuhan, Hubei Province, China, in December 2019 spread rapidly, causing a high number of deaths worldwide. The difficult ability to contain the transmission of the disease raised doubts about the possible forms of contamination. Studies have shown an increase in new cases of the disease on days when the level of pollution was high, raising questions that pollutants may be carriers of the virus. In this study, we investigated the involvement of fine particulate matter (PM_{2.5}) in virus loading in common circulation (indoor and outdoor) environments and in the intensive care unit (ICU) of a hospital. PM_{2.5} was collected from May to November 2020, and the collection time per day was 48 to 72 h. After collection, the material was stored at a temperature of -80°C until the moment of analysis. Our results demonstrated that SARS-CoV-2 can be found in fine particulate material (PM_{2.5}), but there is an essential interference of temperature, humidity and UV rays in the preservation of viral RNA.

Introduction

COVID-19 is a disease caused by the new coronavirus (SARS-CoV-2), which emerged in Wuhan, Hubei Province, China, in December 2019. Initially, the World Health Organization (WHO) was informed by the Chinese government of an outbreak of pneumonia of unknown etiology, and in January, the Chinese National Health Commission already knew it was a pneumonia of viral origin and that from the isolation of the virus in hospitalized patients, the new coronavirus was identified (Wang et al. 2020)(Shereen et al. 2020).

A study developed by Zhu et al. explored the relationship between air pollutants and infection caused by the new coronavirus (Copiello and Grillenzoni 2020). The number of new daily cases, the concentration of pollutants (PM_{2.5}, PM₁₀, SO₂, CO, NO₂ and O₃) and meteorological variables in 120 cities in China were evaluated using a generalized additive linear model. The results showed that there was a positive association of PM_{2.5}, PM₁₀, NO₂ and O₃ in the last two weeks and the number of new cases. An increase of 10 µg/m³ (lag0-14) in PM_{2.5}, PM₁₀, NO₂, and O₃ is associated with increases of 2.24% (95% CI: 1.02 to 3.46), 1.76% (95% CI: 0.89 to 2.63), 6.94% (95% CI: 2.38 to 11.51), and 4.76% (95% CI: 1.99 to 7.52) in the daily number of confirmed cases of COVID-19, respectively. For an increase in the same order of SO₂, there is a decrease of 7.79% in the number of confirmed cases.

SARS-CoV-2 is an extremely small virus approximately 65–125 nm (nanometers) in diameter. It consists of a simple RNA strand covered by a protein shell (capsid) on which there is an envelope, and it has spikes, which give the appearance of a solar crown; its name coronavirus is derived from its appearance (corona = crown in Latin) (Cienciewicki and Jaspers 2007)(Domingo, Marquès, and Rovira 2020).

In 2017, Cienciewicki and Jaspers conducted an epidemiological study on air pollution and viral respiratory infections. They observed that there is a positive correlation between the levels of particulate

matter, cardiovascular mortality and respiratory conditions, suggesting that exposure to PM alters respiratory immunity to viral infections (Ciencewicki and Jaspers 2007).

Another plausible explanation for this association was proposed by Setti and collaborators at the University of Bologna in Italy (Domingo, Marquès, and Rovira 2020). They suggest that air pollution can preserve the viability of the virus, increase its potential for infection and facilitate transmission through interaction with the particulate material present in the air. The particles would act as carriers for the virus, as is already known for other chemical and biological contaminants. Therefore, the particulate material acts as a carrier and substrate for the virus (Weinbauer et al. 2009)(Sedlmaier et al. 2009).

Particulate matter is generated essentially by the incomplete burning of fossil fuels and biomass. It consists of a central core of elemental carbon, and on its surface, other compounds can bond (e.g., heavy metals) (Huffman et al. 2000)(Schlesinger 2007). Its structure also allows the attachment and survival of microorganisms (bacteria, viruses). Studies indicate that biological material comprises a substantial fraction of fine (PM_{2.5}) and coarse (PM₁₀) particles, which may represent 10 to 25% of the mass (Jalava et al. 2015; Samake et al. 2017)(Cardoso et al. 2017).

It is also believed that the biological diversity present in the particulate material is high, with numerous pathogenic and/or nonpathogenic microbial species (viruses, bacteria and fungi) coexisting in the same coarse, fine and ultrafine particles (Acosta-Martínez et al. 2015; Gardner et al. 2012). The pathogenicity and toxicity of these “bioaerosols” will therefore depend on the components of the particulate material, the particle size and the concentration of microorganisms (Samake et al. 2017).

Another study monitoring the presence of pathogenic microorganisms (viruses and bacteria) associated with PM_{2.5} in Seoul air during the “yellow dust” phenomenon and outside this period showed that there was a presence in the particulate material of viruses that caused respiratory infections such as rhinovirus, norovirus and parechovirus (Chung, n.d.; Han et al. 2018).

Although previous studies have already confirmed and shown effective results in detecting the (Gendron et al. 2010; Han et al. 2018; “A Field Indoor Air Measurement of SARS-CoV-2 in the Patient Rooms of the Largest Hospital in Iran” 2020) of viruses in bioaerosols, the main challenge of monitoring the presence of SARS CoV-2 in the air lies precisely in the probable low concentration of the virus in the particulate matter present in the air (Cao et al. 2014)(Hermann et al. 2006). This study first evaluated two sampling methods (air filtration and liquid collision method AGI-30) over long samplings. Additionally, indoor and outdoor sampling was performed to prove this hypothesis, and we investigated the environmental dispersion of SARS-CoV-2 associated with environmental particles.

In this study, we investigated indoor and outdoor air samples using three different methodologies to detect SARS-CoV-2 and whether environmental factors can predict the presence of SARS-CoV-2 RNA in PM_{2.5}

Methods

All methods were performed in accordance with relevant guidelines and regulations and all environmental experimental protocols do not require institutional committee approval. However, for the cough sampling of COVID-19 positive patients, an Informed Consent Form was applied and signed.

1.1. Sampling

Three methods of fine particulate matter ($\leq 2.5 \mu\text{m}$ aerodynamic diameter, PM_{2.5}) collection were used.

The first was a custom-designed virtual Harvard Micro-Environmental Cascade Impactors (Demokritou et al. 2002) (Fig. 1) placed 1.5 m above the floor. For each sampling period, the impactor ran continuously for 24 h at a constant flow rate of 5-7.5 L/min, which corresponds to a total sampling volume of 7.2–10.8 m³. The collected material was retained in filtering membranes with pore sizes of 0.22 μm and 47 mm diameter (cellulose Millipore GSWP04700 and polytetrafluoroethylene hydrophobic vials MFPT-4722).

The second method utilized liquid collision in all glass impingers. In this method, the air is pumped into a glass bottle containing a RNase-free liquid in which the particles are suspended (AGI-30) placed on the floor (Harstad 1965)(Artenstein and Cadigan 1964; Schaffer, Soergel, and Straube 1976) UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen) (Chang and Chou 2011). The volume varied as shown in table. The sampling period was 24 hours, with a flow rate of 1 L/min corresponding to a total sampling volume of 1.44 m³ (Fig. 1).

The third method used for indoor collection at the intensive care unit (ICU) was Dusttrak virtual impactors with a cut for particles of 2.5 μm in diameter (PM_{2.5}) with a flow of 1 L/min for periods of 48 to 72 hours of sampling method selected for sampling the fine particulate material. The equipment was placed 1.2 meters above the floor. It was located in the passage of ICU employees and inside patient rooms.

Samples collected using those three methods were stored at -80°C until the time of analysis.

1.2. Air sampling location

1.2.1. Indoor and Outdoor of The Faculty of Medicine of the University of São Paulo

Environmental samples were collected from May to November 2020 in external and internal places with high circulation of people and air pollution exposure near The Faculdade de Medicina da Universidade de São Paulo (FMUSP), located in the perimeter of the largest hospital of South America, Hospital das Clínicas (Fig. 2). The collecting devices were located A) near access to the FMUSP across Av. Dr Arnaldo, which has a high flow of automobiles, in front of Clinicas subway station, B) central floor of the FMUSP building, C) pedestrian crossing on Rua Dr. Eneas, which has a large circulation of patients and employees of Hospital das Clínicas.

1.2.2. Indoor-intensive care unit (ICU) dedicated to COVID-19 patients

From November to December 2020, collections were made indoors at the ICU, in patients' rooms and nurse's desk (Fig. 3), located in 11th Floor of Hospital das Clínicas, that exclusively serve COVID-19-positive patients (Fig. 3). To validate this method, a patient newly diagnosed with COVID-19 was invited to participate in the research, being informed of the objectives of this project and providing his free and informed consent. We used a sample collected after a patient coughed over the air suction nozzle of the Dustrack impactor.

1.3. RNA extraction and RT-qPCR

Initially, the filters were placed in 2 mL tubes and incubated for 20 minutes with 250 µl of PBS under vigorous and constant agitation in a vortex (Vortex-Genie 2). Viral RNA was extracted using TRIzol® LS reagent (Invitrogen); 750 µl of TRIzol® LS was added, and the mixture was vortexed and incubated for 3 hours at 4°C. After this, the extraction procedure followed the manufacturer's protocol. The RNA pellet was resuspended in 50 µl of sterile RNase-free water. Nucleic acid from material obtained through air bubbling was extracted from 250 µl of the solution following the same procedure described above.

Molecular detection of SARS-CoV-2 was performed using the SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen) with primers and probes described in the CDC protocol that amplify the region of the nucleocapsid N gene (2019_nCoV_N1 and 2019_nCoV_N2 assays) and reverse-transcription quantitative real-time polymerase chain reaction (RT-qPCR) analysis. The reactions were carried out in a 7500 Fast Real-Time PCR System (Applied Biosystems) and consisted of a step at 50°C for 15 minutes for reverse transcription, followed by incubation at 95°C for 2 min and 45 cycles of temperature varying from 95°C for 15 seconds to 55°C for 30 seconds.

Division of Viral Diseases. 2020. 2019-novel coronavirus (2019-nCoV) real-time rRT-PCR panel primers and probes. Centers for Disease Control and Prevention, Atlanta, GA ("rRT-PCR Panel Primers and Probes" n.d.)

1.4. Environmental data collection

Temperature and humidity data of the investigated region were obtained from the Sao Paulo State Environmental Company (CETESB), and UV index (RADUV) data were collected in the Temis UV index. ("TEMIS v2.0 UV Index" n.d.)

1.5. Statistical analysis

Binary logistic regression was performed to verify the association of the environmental factors with the positive detection of the SARS-CoV2 virus.

Results And Discussion

Analysis of the environmental variables collected alongside the 198 fine size fraction samples showed that humidity was a significant predictor of viral RNA presence in the collected samples (OR = 0,801; CI 95%=0,651-0,985, $p = 0,036$). We did not observe such associations with temperature and UV radiation. A summary of the environmental data is shown in Table 1.

Table 1
Descriptive analysis of the variables used in the study that consisted of 198 samples.

	N	Min	Max	Mean	Sd
Humidity (%)	198	21,0	80,0	39,4	13,9
Temperature (°C)	198	17,2	37,1	27,3	3,8
RADUV (W/m2)	198	8,0	46,0	32,4	8,3

2.1. Comparison of sampling methods for SARS-CoV-2 detection in indoor and outdoor samples

2.1.1. FMUSP indoor and outdoor sampling

From May to October 2020, 103 samples were collected in the same place on a filtering membrane in the cascade impactors, and another 56 samples were collected using the liquid collision method for all glass impingers using the bubbling of air in the liquid.

Collection of the samples using a filtering membrane did not allow for successful detection of the virus RNA using RT-qPCR in any of the collected samples. While collection of the samples using liquid medium enabled us to detect, using RT-qPCR with N1 primers/probe set SARS-CoV-2 RNA was detected in 7 samples and N2 primers/probe set in 1 sample from 56 collected. According to the Emergency Use Authorization (EUA), detection of either the N1 or N2 gene is considered positive for the presence of SARS-CoV-2 ("Orig3n 2019 Novel Coronavirus (COVID-19) Test EUA Summary" n.d.). We detected positive results for SARS-CoV-2 genes in the aqueous sample, as shown in Table 2. The cycle threshold (CT) value was set as < 40 to confirm the positive detection of SARS-CoV-2. Further comparison between liquid impingement (method I) and air filtration methods (method II) indicates that sampling by AGI-30 with DW was the most appropriate for virus detection. Nevertheless, a major disadvantage of conventional impingers (AGI-30, method II) is the loss of collection fluid during sampling because of violent bubbling (Van Droogenbroeck et al. 2009), which can reduce microbial recovery due to reaerolysis (Lin et al. 2000). The adverse effects of prolonged sampling may be partially responsible for the low detection of virus.

2.1.2. ICU Indoor sampling

Among the 25 collections performed using Dustrack impactor filtering membranes in the ICU, in two samples, the N1 nucleocapsid gene of SARS-CoV-2 RNA was successfully detected by RT-qPCR. Only in

one of these samples did we also detect the N2 gene. One of the positive samples was inside one of the patient's rooms and the other at the nurse's desk in front of the main aisle (Table 2). The singularity of this result is that viral RNA could still be detected in the ICU of individual occupations and in the place of passage of the employees. The degree of viral spread (from patients) may be due to symptoms such as coughing and sneezing (Schijven et al. 2021). It has been suggested that the presence of virus particles in a poorly ventilated environment may increase the dispersion of the virus (Morawska and Cao 2020; Qian, Ferro, and Fowler 2008). The sampling time (48–72 hours) was sufficient to detect viral RNA in this study (Copat et al. 2020).

Table 2
Positive results for SARS-CoV-2 genes in the aqueous sample and Dustrack air samples.

Sample ID	Local Description	Sampling data	Cycle threshold N1 gene	Cycle threshold N2 gene
Membrane Filter Control to Dustrack	Covid positive patient cough	flow: 1,5 l/min, PM 2.5 Filter PTFE	36	Negative
7	Nurse's Desk ICU	flow: 1 L/min, PM 2.5 Filter PTFE	37,4	Negative
6	Dustrack ICU FF07 (bed 07)	flow: 1 L/min, reducer to PM 2.5 Filter PTFE	36,5	36,7
33	AGI-30 FMUSP Entrance (A)	Aqueous Sample Volume 4 ml	36,9	Negative
32	AGI-30 FMUSP Building (B)	Aqueous Sample Volume 6 ml	37	Negative
44	AGI-30 FMUSP Building (B)	Aqueous Sample Volume 3 ml	37	37,5
35	AGI-30 FMUSP Building (B)	Aqueous Sample Volume 3,5 ml	36,9	Negative
9	AGI-30 FMUSP Entrance (A)	Aqueous Sample Volume 6 ml	36,9	Negative
8	AGI-30 FMUSP Building (B)	Aqueous Sample Volume 6,5 ml	37	Negative
13	AGI-30 Security Dr. Eneas St. (C)	Aqueous Sample Volume 4,5 ml	36,9	Negative

Conclusion

The present study provides a comprehensive assessment of the three methodologies used to detect SARS-CoV-2 in air samples. We found that liquid collision in all glass impingers, AGI-30, was the best sampling method for SARS-CoV-2 detection environments. However, the number of cycle threshold N1 genes was so high that it was indicative of a low viral load (Table 2). We explain the lack of virus detection by the virtual Harvard Micro-Environmental Cascade Impactors method in the outdoor environment due to the high sensitivity of the virus to changes in temperature, humidity and environmental UV rays that can cause the degradation of the virus RNA (Nicastro et al., n.d.; Ratnesar-Shumate et al. 2020; Sagripanti and Lytle 2020). This shows that the viral particles can be associated with PM_{2.5}. However, in outdoor environments, the influence of climatic factors can affect viability (Robotto et al. 2021). However, the fact that there is no positivity for SARS-CoV-2 in PM_{2.5} does not mean that it is not necessary to decrease it as a transport of pollutants (Wu et al., n.d.). Mainly PM₁₀ and PM_{2.5} are particles highly associated with illness in the population (Manisalidis et al. 2020; Anderson, Thundiyil, and Stolbach 2012)(Kampa and Castanas 2008).

While samples were collected in closed environments with controlled temperature and humidity, positivity detection for air filtration sampling (Dustrack) is suitable for capturing viruses in indoor ICU environments.(Md et al., n.d.) followed by analysis with qPCR.

The presented data can help to improve the accuracy of the assessment of airborne SARS-CoV-2 exposure in closed environments. This is particularly important in the context of recent findings showing that surfaces present relatively little risk of transmitting the virus, while pressure on controlling air quality in closed environments should be prioritized (Lewis 2021a)(Lewis 2021b)(Agarwal et al. 2021). A suitable method for air sampling can help track the most aerosolization potential SARS coronavirus-2 in determining the propagation of contaminated sources. Efficient air sampling and appropriate analysis will be beneficial in preventing the spread of the disease.

Declarations

Author contributions:

M.M.V. and N.V.S. designed research; N.V. S and D.W. collected samples; M. G, J.R.P., A.S.S. L and Y.H. developed to methods to analyze samples; M.M.V. performed the statistical analysis; M.M.V., N.V.S., and Z.W. wrote the draft manuscript; all authors revised the paper writings.

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Figures

Figure 1

Schematic illustration of the equipment used to collect the air samples to monitor COVID-19.

Figure 2

Air sampling locations in FMUSP. A) near access to the FMUSP across Av. Dr Arnaldo, which has a high flow of automobiles, in front of Clinicas subway station, B) central floor of the FMUSP building, C) pedestrian crossing on Rua Dr. Eneas, which is a large circulation of patients and employees of Hospital das Clinicas

Figure 3

Indoor-COVID-19 ICU: Localization of the air suction nozzle of the Dustrack impactor in the indoor air sampling location at the intensive care unit (ICU) dedicated to COVID-19 patients and in Nurse's desk.