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Detection of Sars-Cov-2 in the air of two hospitals in Hermosillo, Sonora, México, utilizing a low-cost environmental monitoring system

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ABSTRACT

Objective: The best way of preventing the dispersion of an infectious disease is decreasing the transmissibility of the pathogen. To achieve such a goal, it is important to have epidemiological surveillance to retrieve data about its routes of transmission and dispersion. This study investigated the possibility of SARS-CoV-2 detection using filtration through 0.22 μm pores.
Methods: A filtration system with vacuum pump was used for sampling, and molecular analysis was performed by RT-PCR for detecting the COVID-19 virus.
Results: It was found that SARS-CoV-2 could be detected in particulate matter trapped on 0.22 μm filters 3 h after air sampling, and the only contaminated areas were those near patient zones.
Conclusions: The results confirm the possibility of finding this virus in floating particulate matter in contaminated zones, with a simple and economic sampling method based on filtration technology through 0.22 μm pores and detection with molecular techniques (RT-PCR). The higher risk zones were those near patients with COVID-19.

Introduction

One of the main strategies to prevent the spread of respiratory diseases is by reducing person-to-person transmission. This can be accomplished by restricting direct contact between patients and close contacts. However, this approach is difficult to achieve, because, in most of the cases, individuals who carry respiratory diseases do not have clinical manifestations in the early stages. Modern transportation systems also facilitate dissemination of pathogens from one place to another. The spread of pathogens from one site to another and their transference between individuals generally occurs through close human contact, which can lead to social and economic disarray (Gray, 2020). In addition to personal transmission, there is a high risk of pathogens spreading through the air. Sneezing and coughing once can produce up to 3000 droplets; such particles can fall over people, clothes and surfaces, facilitating spread of a virus. Moreover, some small particles can remain in the air (Li et al., 2020) and bind to environmental particulate matter (PM), adsorbing and accumulating microorganism over surfaces (Santos-Romo et al. 2014, 2019), which can be disseminated by the wind.

At the end of December of 2019, China notified the world of an outbreak of an unknown pneumonia in Wuhan. Later, the causative agent was identified as a new type of coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since then, the disease was labeled by the World Health Organization as 2019 coronavirus disease (COVID-19) (Khan, 2020). It has been documented that SARS-CoV-2 can remain infective on surfaces like

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wood, glass, metal, etc. (Kampf et al., 2020; Van Doremalen et al., 2020). Viruses can contaminate surfaces through small saliva droplets directly deposited on different surfaces or due to a process of sedimentation of PM containing the virus.

It has also been recognized that SARS-CoV-2 can be transmitted through fecal matter (Amirian, 2020; Wang et al., 2020a, 2020b; Xiao et al., 2020; Zhang et al., 2020a, 2020b), making transmission through the fecal-oral route plausible. Previous research on other coronaviruses, including SARS and MERS, has shown that surfaces can be efficiently disinfected by solutions prepared with 0.1% sodium hypochlorite or 70–78% ethanol, which significantly reduces their infectivity (Fathizadeh et al., 2020; Kampf et al., 2020).

The high transmissibility shown by SARS-CoV-2 has caused scientific uncertainty, as well as social and sanitary challenges all over the world, making design strategies for prevention and control of spreading of the utmost importance. An initial step to mitigate the dissemination of SARS-CoV-2 is accurately identifying its location. Recent studies on the permanence of SARS-CoV-2 in environmental air have not been conclusive; although there is some evidence that the virus can remain in the air by being bound to PM (Chen et al., 2020; Zhang et al., 2020a, 2020b).

Due to the risk of respiratory contamination, analysis of air in health facilities and mass population assistance sites seems to be a good alternative to monitoring the presence of SARS-CoV-2, which can help in disinfection of contaminated areas to decrease transmission of the virus. To guarantee efficacy of this approach, it is necessary to have efficient sampling and diagnostic techniques to timeously detect the presence of the virus in environmental PM and also in healthy carriers and inert contaminated surfaces (Guo et al., 2020). This methodology can be used in hot spots for risk transmission like hospitals and healthcare facilities.

Previous research carried out in Hermosillo, Mexico, documented bacterial contamination in the air by using traditional and molecular biology techniques in PM 10 and PM 2.5. Fecal bacteria were found utilizing filters for particles PM 10 and PM 2.5 (Santos-Romo et al., 2014, 2019). These techniques can be applied for detecting viruses. This study aimed to implement an environmental surveillance system utilizing vacuum-assisted filtration membranes with 0.22 μm pores to search for the presence of SARS-CoV-2 in the air environment of two hospitals in the city of Hermosillo, Sonora, México. This city has 1 million inhabitants, and the highest attack rate, incidence, morbidity, and mortality by COVID-19 within the state of Sonora.

Methods

Environmental sampling

A vacuum pump was used to sample the air in three areas of Clinic A and the COVID-19 patients care room of Clinic B. These areas were named Emergency area (Clinic A), Internal medicine (Clinic A), COVID area (Clinic A), and COVID-19 patients care room (Clinic B). Sampling in all areas was accomplished in 3 h. Filters of 25 mm diameter with 0.22 μm pores were utilized (Millipore, AAWP02500), placed in a sterilized filter holder (Millipore, SWINNX) coupled to a vacuum system through a previously disinfected plastic hose (Figure 1), filtering the air with a flow of 9.6 L/min in each filter holder.

Isolation of viral RNA

Filters were removed from the samplers and placed in a 1.5 mL tube containing lysis solution (Qiagen kit or LUSIGEN kit). The filter holder and hoses were disinfected with 70% alcohol solution according to the protocol of Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/prepare/cleaning-disinfection.html). The filters were macerated using nuclease-free disposable plastic pistils to obtain total RNA. For the diagnosis of SARS-CoV-2, total RNA was utilized through a real-time reverse transcription-polymerase chain reaction (RT-PCR) technique. The RNA was extracted applying a commercial kit (RNAspin miniRNA Isolation kit, G.E. 25-0500-71) following the manufacturer's instructions or with a fast method (QuickExtractTM DNA Extraction Solution, LUCIGEN Symplifying Genomics, QE0905 T), placing 50 μL of the sample in 50 μL of the reagent, followed by an incubation of 5 min at 95 °C, and then cooling it with ice (Ladha et al., 2020; Moreno-Contreras et al., 2020). The extracted material was utilized for amplification by RT-PCR specifically for SARS-CoV-2 and it was analyzed in the Laboratorio

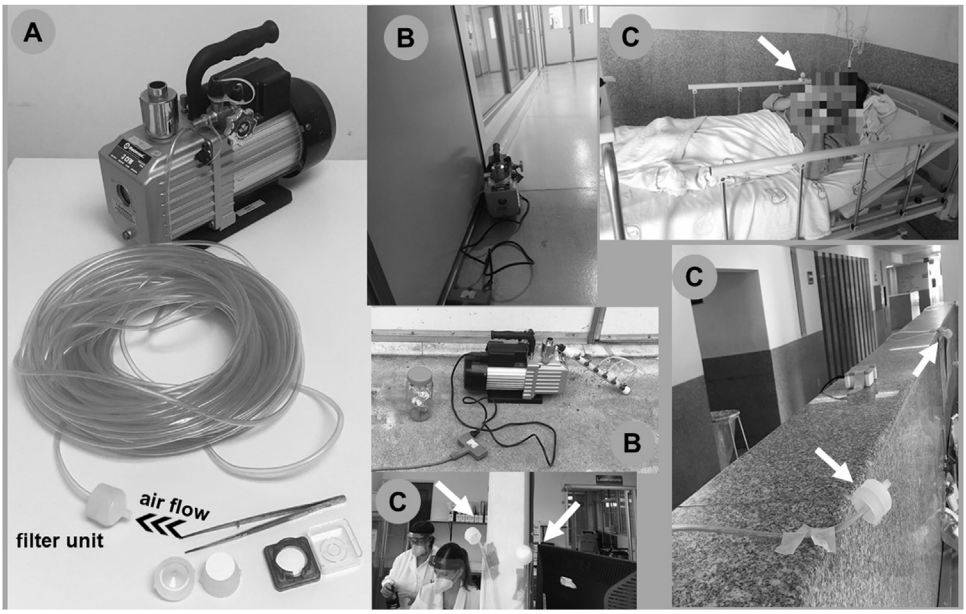

Figure 1. (A) The filtration air device. The air flow that was generated by the vacuum pump was of 9.6 L/min. (B) The pump system working in hospital areas. C) Air filter units in different hospital areas (white arrows).

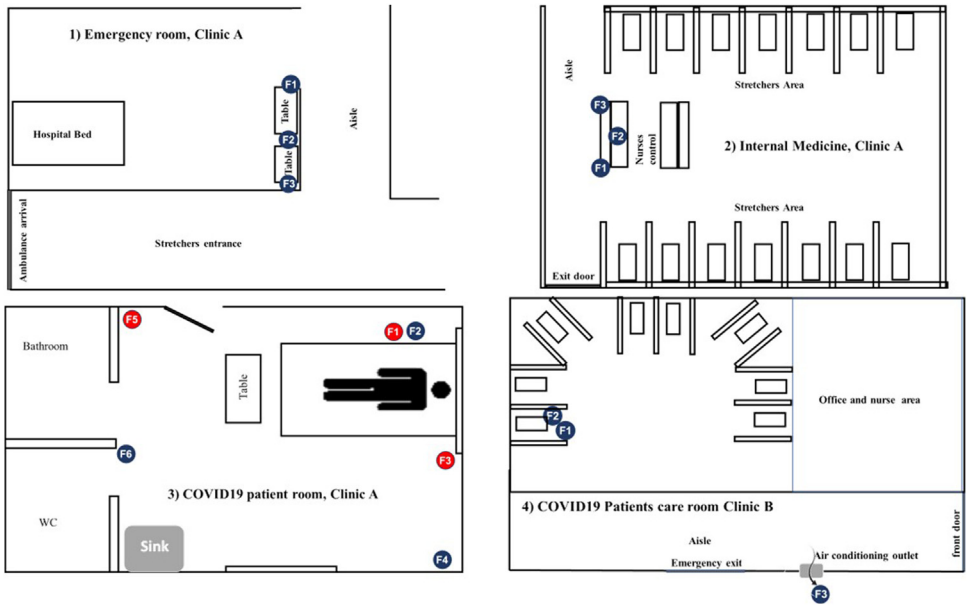

Figure 2. The areas sampled in the two hospitals. The RT-PCR technique was used to detect SARS-CoV-2 in the air. Red circles indicate air samples with the presence of the virus, blue circles indicate negative samples. Filter F3 in the COVID-19 patients care room in Clinic B indicates the air outlet from the COVID area.

de Referencia, Análisis y Diagnóstico en Sanidad Acuícola (LADARSA) from the CIBNOR unit located in Hermosillo, Sonora, México.

Bacterial count in sampling areas

An additional filtration unit was placed in each sampled area for bacterial counting, as a punctual contamination marker. The UFC count was recorded, as by Nobile (1967) and Hernández-López et al. (1995), with some modifications. In brief, 22 mm diameter filters with 0.22 μm pores and petri plates with tryptic soy agar (TSA) containing 0.1% of tetrazolium violet were used and incubated for 24–48 h at 35 °C. Colony forming units (CFU) were counted and the bacteria that were obtained were analyzed by colonial morphology, Gram staining and biochemical reactions.

Reverse transcription and RT-PCR

The RNA was processed by real-time RT-PCR with commercial reagents (WoV19 Kit), according to the protocol described by Corman et al. (2020), which included: RdRp_SARSr-F 5′-GTGARATGGTCATGTGTGGCGG-3′ and RdRp_SARSr-R 5′-CAR-ATGTTAAASACACTATTAGCATA-3′ primers that amplify a 100 pb fragment of the viral gene of the RNA polymerase and a probe FAM-CAGGTGGAACCTCATCAGGAGATGC-BHQ; the E_Sarbeco_F 5′-ACAGGTACGTTAATAGTTAATAGCGT-3′ and E_Sarbeco_R 5′-ATATTGCAGCAGTACGCACACA-3′ primers that amplify a 113 pb fragment of the envelope protein gene of SARS-CoV-2 and a probe FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1. The primers

F 5′-AGATTTGGACCTGCGAGCG-3′ and RP-R 5′-GAGCGGCTGTCTC-CACAAGT-3′ and probe TTCTGACCTGAAGGCTCTGCGCG-BHQ1 that amplify a 65 pb fragment of the human *RNasaP* (RP) gene were used as an internal control. As positive standard was used: overexpressed

Table 1
Results of bacterial culture in different zones of Clinic A and Clinic B from Hermosillo, Sonora.

Sample area	Filtration unit	CFU	Isolation	Biochemical characteristics
Emergency room Clinic A	Filter 3	9	*Streptococcus spp.*	Gram-positive cocci in short chains, anaerobic and lactose fermentative, catalase (-), motility (-), nitrate reductase (+) and alpha-hemolysis (+)
			Bacillus spp.	Gram-positive aerobic rods in single and short chains, catalase (+), motility (+) and nitrate reductase (+)
Internal Medicine Clinic A	Filter 3	35	*Staphylococcus spp.*	Gram-positive cocci in clusters, facultative anaerobic, lactose fermentation (+), catalase (+), motility (-), coagulase (+)
			Micrococcus spp.	Gram-positive aerobic non-fermentative cocci in irregular clusters, catalase (+), motility (-) and nitrate reductase (-)
COVID-19 patient room Clinic A	Filter 2	40	*Bacillus spp.*	Gram-positive aerobic rods in single and short chains, catalase (+), motility (+) and nitrate reductase (+)
			Micrococcus spp.	Gram-positive aerobic non-fermentative cocci in irregular clusters, catalase (+), motility (-) and nitrate reductase (-)
	Filter 6	10	*Streptococcus spp.*	Gram-positive cocci in short chains, anaerobic and lactose fermentative, catalase (-), motility (-), nitrate reductase (+) and alpha-hemolysis (+)
			Staphylococcus spp.	Gram-positive Cocci in clusters, facultative anaerobic, lactose fermentation (+), catalase (+), motility (-), coagulase (+)
COVID-19 patients care room Clinic B	Filter 2	15	*Streptococcus* spp.	Gram-positive cocci in short chains, anaerobic and lactose fermentative, catalase (-), motility (-), nitrate reductase (+) and alpha-hemolysis (+)

CFU, colony forming units.

Table 2
Results of the RT-PCR analysis for detecting SARS-CoV-2 in air samples from three areas of Clinic A and one area of Clinic B in Hermosillo, Sonora.

Sample area	Filtration unit	RT-PCR results
Emergency room Clinic A	Filter 1	ND
	Filter 2	ND
	Filter 3	Used for bacterial count
Internal medicine Clinic A	Filter 1	ND
	Filter 2	ND
	Filter 3	Used for bacterial count
COVID-19 patients room Clinic A	Filter 1	**D**
	Filter 2	Used for bacterial count
	Filter 3	**D**
	Filter 4	ND
	Filter 5	**D**
	Filter 6	Used for bacterial count
COVID-19 patients care room Clinic B	Filter 1	ND
	Filter 2	Used for bacterial count
	Filter 3	ND

D, Detected; ND, Not detected.

mRNA from SARS-CoV-2, provided by the Instituto de Biotecnología de la UNAM (IBT)-Consejo Nacional de Ciencia y Tecnología (CONACyT), México. The RNA control for the constitutive gene was obtained from human blood cells extracting ARN from peripheral blood of a SARS-CoV-2-negative person using RNAspin miniRNA Isolation kit, G.E. 25-0500-71, and it used a negative sample provided by the Sonora State Laboratory (LESPS) as a negative control.

Results

This study used the bacterial counts in hospital areas only as overall references of contamination and only one filter in each area was used for this purpose. The bacterial growth analysis showed different colonial morphology in TSA plates; the outcomes of the samples analyzed for bacteria are shown in Table 1. According to the definition of clean areas (Caorsi et al., 2011), the number of bacteria did not exceed the cleaning standards in any of the analyzed samples.

All of the samples for molecular analysis were set in a lysis solution before taking them out of the sampled hospitals, to prevent the introduction of infectious material in the CIBNOR facilities. The preparation of materials and equipment was performed inside the hospitals before initializing the sampling, and sanitization of all materials used was performed before leaving the hospitals.

The sampling units were distributed in such a way that the presence or absence of the virus could be demonstrated near the patients and in some places far away from them. The distribution of the filters, according to their position in each area, is shown in Figure 2.

The sampling in all areas was accomplished in 3 h, after which, the filters were removed and placed inside the lysis solution for the extraction of viral RNA. In each sampled area, a filter was used to search for total bacteria. The results are shown in Table 2. The distribution of positive and negative samples of the analyzed areas is shown in Figure 2.

Discussion

According to the reports by Santos-Romo et al. (2014, 2019), the presence of bacteria in environmental PM is a biological quality marker of the air; therefore, the environmental bacterial count can be an indirect measurement of good or bad air quality in a given setting. This study used air bacterial counts to define the cleaning of the sampled areas. A low bacterial count was seen in the air of each analyzed area in the clinics, allowing them to be defined as clean zones, according to Caorsi et al. (2011).

This study used a low-cost method to obtain viral RNA, without the necessity of complicated extraction processes, using the LUCIGEN kit (Ladha et al., 2020; Moreno-Contreras et al., 2020), which contributed to decreased time and cost for RNA extraction; it also reduced the production of waste, like tubes and columns, produced by the extraction with other commercial kits.

There is some evidence that SARS-CoV-2 is transmitted by air, but there are inconclusive results (Faridi et al., 2020; Ghinai et al., 2020; Holshue et al., 2020; Van Doremalen et al., 2020). It is known that the virus is found in saliva droplets and has the capacity to remain for a variable time on different materials (Van Doremalen et al., 2020; Wu et al., 2020a, 2020b). The current results are in accordance with those published about the search for SARS-CoV-2 in the environmental air, showing that the virus can be found in the air in the hospital zones where COVID-19 patients are placed (Faridi et al., 2020; Wang et al., 2020a, 2020b; Wu et al., 2020a, 2020b). Different systems have been used to acquire air and surface samples, with the use of swabs (Wang et al., 2020a, 2020b), passive precipitation (Wu et al., 2020a, 2020b), gelatin filters (Ong et al., 2020; Van Doremalen et al., 2020), pumped into a sterile impinger containing 20 mL agar DMEM (Dulbecco's Modified Eagle's Medium) with 100 μg/mL streptomycin, 100 U/mL penicillin (Faridi et al., 2020), and polytetrafluoroethylene filters with 0.3 μm pores (Ong et al. 2020). Although the use of filters is common for assessing air quality, a handful of studies have used this matrix for their biological pollution research of air samples (Santos-Romo et al. 2014, 2019).

The current study demonstrated the efficiency of filtration units with a vacuum pump containing filters with 0.22 μm diameter pores for detecting SARS-CoV-2 in environmental PM. This sampling system took PM in a controlled environment, filtering air with a flow of 9.6 L/minute from selected areas. A recent report with a similar method for searching SARS-CoV-2 in air used 0.3 μm pores and filtration with a flow of 5 L/min (Ong et al., 2020). Faridi et al. (2020) used the vacuum system with a pressure of 1.2 L/min, but they did not use a filter for retaining contaminated particles. Considering that a virion of SARS-CoV-2 is about 80 nm (0.08 μm), using pores > 0.08 μm does not ensure that the individual virions are going to be captured in the filter. Nevertheless, knowing the dynamic of microorganisms in the environment, virions can stick to the PM in the environment that generally presents with sizes between 1–10 μm (PM 10, PM 2.5 and PM 1.0). If the viruses can

maintain themselves in the PM that is found in the air, then transmission can be possible for a longer time and distances than the ones calculated for the surfaces.

In this study, the bacterial growth in the analyzed samples indicated that the filter trapped air PMs > 0.22 μm. In the same way, the detection of SARS-CoV-2 in some areas near COVID-19 patients showed the utility of this system for detecting SARS-CoV-2. The results of the samples from two hospitals in the city of Hermosillo, Mexico, showed that the areas close to the patients (between 0.5–2 m) were contaminated by SARS-CoV-2, while places further away did not have presence of the virus. These observations are consistent with those reported by Faridi et al. (2020), who found that all air samples that were collected from 2 to 5 m from the beds of patients with confirmed COVID-19 were negative. These observations suggest that there should be an increase in sanitary protection near the patients, due to the high possibility of transmission caused by aerosolized particles. The implemented system was proven to be efficient in acquiring the SARS-CoV-2 virus and for detecting contaminated areas, depending on the distance of positive patients. Also, this filtration system is low cost and easy to control without the necessity of trained staff.

These results provide an important perspective into implementing strategies for prevention and control of viruses due to the detection of SARS-CoV-2 in air particles in different wards inside hospitals, and targeting sanitization of the areas. Reducing healthcare workers to virus exposure could also help to improve sanitary conditions for COVID-19 hospitalized patients. It is hoped that with the implementation of new actions to prevent the spread and transmission of SARS-CoV-2 through environmental contaminated particles, significantly decreasing the transmission of the virus could reduce the incidence of patients getting it and expenditure for healthcare attention, treatments, hospitalization, and caring for these individuals.

Based on these results it is proposed that this methodology has many advantages and possibilities of being used in any type of hospital, including those with low resources, since it does not require huge expense, specialized personnel, or an area with a high population to implement preventive sanitation actions. It can also decrease the health risk for healthcare workers and the general population.

Conflict of interests

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.

Ethical approval

Not required.

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