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Short Communication

Evidences of SARS-CoV-2 virus air transmission indoors using several untouched surfaces: A pilot study

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HIGHLIGHTS

• There is a controversy about the routes of COVID-19 transmission.
• “COVID traps” have been developed to measure the capacity of SARS-CoV-2 aerosol transmission.
• The detection method is exactly the same for patients and for surfaces trapped in “COVID traps”.
• The COVID-19 air transmission indoors has been demonstrated using these traps.
• These data support the recommendation to carry out frequent disinfection of the surfaces of hospitalized patients.

GRAPHICAL ABSTRACT

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ABSTRACT

Nowadays, there is an important controversy about coronavirus air transmission. The aim of this study was to determine aerosol transmission from patients with coronavirus infection using “COVID-19 traps” that included different untouched surfaces within them. 42 swab samples of 6 different surfaces placed in the rooms of 6 patients with a positive diagnostic of COVID-19 were analyzed with RT-PCR technique to evaluate the presence of the virus and its stability. Samples were collected at 24, 48 and 72 h. Patients were in an intensive care unit (ICU) and in a COVID-19 ward unit (CWU) at a Spanish referral hospital. None of the samples placed in the ICU unit were positive for COVID-19. However, two surfaces, placed in a CWU room with a patient that required the use of respiratory assistance were positive for coronavirus at 72 h. Surfaces could not be touched by patients or health workers, so viral spreading was unequivocally produced by air transmission. Thus, fomites should be considered as a possible mode of transmission of coronavirus and frequent disinfection of surfaces should be taken into account. Our results, although preliminary, point the importance of SARS-CoV-2 virus air transmission indoors and may shed some light in this debate.

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1. Introduction

A novel human coronavirus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (also called COVID-19) emerged in Wuhan, China, in late 2019 followed by East Asia, the Middle East, Europe, North and South America, Africa and Oceania causing a pandemic disease with more that 11 million total cases and over 530,000 total deaths in 218 different countries, at the end of June 2020 (Roylab Stats, 2020). Along with social distancing, the use of mask and hand washing has been advised repeatedly as key actions to reduce transmission of the SARS-CoV-2 virus, responsible for the COVID-19 pandemic (Brauer et al., 2020).

However, there is an emerging controversy about the routes of SARS-CoV-2 virus transmission. It has been observed that droplet and inhalation transmission routes predominate over the contact route, contributing 35%, 57%, and 8.2% respectively, on the probability of infection, without use of personal protective equipment (Jones, 2020).

Coronaviruses have been implicated in nosocomial outbreaks with environmental contamination as a route of transmission (Dowell et al., 2004). Similarly, transmission and stability of SARS-CoV-2 has been reported (van Doremalen et al., 2020). Previously, it has been observed that the predicted concentration of SARS-CoV-2 in the air of the patient room is low (<1 gene copy per m² on average), and likely below the limit of quantification for many air sampling methods (Jones, 2020). For that reason we developed this novel technique based on “COVID-19 traps” to measure the capacity of SARS-CoV-2 aerosol transmission. Different surfaces were incorporated into these “COVID-19 traps” to avoid that patient or healthcare personnel could touch these surfaces but allowing air contact at all times. “COVID-19 traps” were placed in an intensive care unit (ICU) and in a COVID-19 ward unit (CWU) and virus quantification was carried out using the same method established for patients, that is, a specific RT-PCR reaction targeting 3 different genes.

2. Material and methods

2.1. Surfaces

Our data consisted on 6 different surfaces trapped in boxes with plastic, protective grids to avoid that samples could be touched by the patient or by the healthcare personnel (Fig. 1). Surfaces of 100 cm² were included into the box and placed 1 m of distance from patients. The different surfaces were: polypropylene (PP), glass, polyvinyl chloride (PVC), methacrylate, agar medium and carbon steel. PP surfaces were obtained from PP black panels and had a semi-gloss finish with a thickness of 2 mm. These PP surfaces had a one-side plastic cover to be removed remove prior to use, ensuring a clean/non-manipulated surface. Glass surfaces had polished edges and were manufactured according to UNE-EN ISO 1514. They had 4 mm of thickness. PVC surfaces were used from dark grey PVC panels with a thickness of 2 mm. These PVC panels had also a one-side plastic cover to be removed remove prior to use, ensuring a clean/non-manipulated surface. Methacrylate surfaces were totally colorless and transparent, with a thickness of 4 mm and obtained from Plexiglas® XT. Carbon steel panels had low carbon content and were manufactured according to UNE 36086/75-I. They had 0.8 mm of thickness. These carbon steel surfaces were covered with grease to avoid oxidation in contact with air.

Surface samples were collected at three different time points (24, 48 and 72H) using nylon swabs immersed in viral transport medium (VTM) (UTM-Copan®) before sampling. Surfaces were placed in pairs in rooms of patients with confirmed COVID-19 infection. 3 of these rooms were in an ICU and 3 in a CWU. In addition, 3 more surfaces were placed in the entrance of the ICU and 3 in the hall of the CWU as negative controls. Samples were taken moving the swab horizontally, vertically and transversely across the sampling area. The swab was immediately placed into 1 mL of VTM and stored at −80 °C until analyzed.

Samples from patients were extracted the day when “COVID-19 traps” were placed in their rooms. Additionally, samples from patients were extracted using the same nylon swabs immersed in VTM than the ones used for surfaces (UTM-Copan®).

2.2. RNA extraction

Patients and surfaces samples were treated equally during RNA extraction and RT-PCR technique. RNA extraction was performed using the automatized system Nuclisens EasyMAG® (bioMérieux) based on the ability of silica to bind DNA and RNA in high salt concentrations (Boom technology). In brief, during incubation of the lised samples, all the target nucleic acids were captured by silica magnetic particles. This way, the Nuclisens EasyMAG® magnetic device attracts all the magnetic silica, enabling the system to purify the nucleic acids through several washing steps. Then, samples were heated, thus releasing the nucleic acids from silica. Finally, the magnetic silica particles were separated from the nucleic acids by a magnetic device and samples were eluted in 50 μL of elution buffer.

Fig. 1. Image of two “COVID-19 traps”. The left one is empty; whereas the right one has a surface inside that cannot be touched. This surface was later tested with nylon swabs moving them horizontally, vertically and transversely across all the sampling area. Immediately, the swab was immersed in viral transport medium and stored at −80 °C until analyzed.
2.3. RT-PCR

The purified RNA was subjected to amplification by RT-PCR (Allplex™ 2019-nCoV Assay®, Seegene). The CFX96 (Biorad®) platform was used for the amplification process following the scheme included in Table 1.

Allplex™ 2019-nCoV Assay is a multiplex RT-PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP (RNA dependent-RNA polymerase) and N (Nucleocapsid) genes specific for SARS-CoV-2, and E (Envelope) gene for all of Sarbecovirus including SARS-CoV-2. The results were analyzed using the software Seegene Viewer V3.18.005.003.

The Allplex™ 2019-nCoV Assay includes a full process internal control which is composed of MS2 phage genome. This internal control material verifies all steps of the analysis process, including sample extraction, reverse transcription, and PCR to demonstrate proper specimen processing and test validity of each specimen.

3. Results

“COVID-19 traps” were placed only in the rooms of patients with a confirmed positive diagnostic. Interestingly, the rooms where COVID-19 patients were isolated had a ventilation rate of 1800 m³/h. This means that the air of the room was completely renovated 50–60 times per hour. Importantly, the air came 100% from the outside of the hospital. It is important to remark that despite the fact that the air of the room was completely renewed every minute, two positives were found in the surfaces trapped in the “COVID-19 traps”, thus showing the infective capacity of SARS-CoV-2 virus on fomites.

As observed in Table 2, none of the surfaces placed in the ICU were positive irrespectively of the Ct of the patient. This fact could be explained because all patients were intubated, and consequently, these patients produced fewer aerosols. As expected, all negative controls were also negative for SARS-CoV-2 virus detection.

Regarding patients in the CWU, two positives were found in two different surfaces (glass and PP) at 72 h, both placed in the same room. Importantly, although another patient in the CWU had similar Cts (Table 2) only the surfaces placed next to the patient with a nasal cannula were positives. Interestingly, positives were only found at 72 h, possibly because an accumulation of virus in time was necessary and as observed in previous studies, virus are stable for more than 72 h at room temperature with constant relative humidity (van Doremalen et al., 2013). In one of the first studies analyzing the stability of SARS-CoV-1 in different surfaces, viruses were dropped onto the surfaces of the testing materials and maintained at room temperature (Duan et al., 2003). Higher infectivity was found in glass, metal and clothes, where they maintained their stability for more than 72 h. In 2013, during the outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV), its stability in several surfaces was also tested under different environmental conditions (van Doremalen et al., 2013). MERS-CoV could still be recovered in its infectious form after 60 h at 20 °C and 40% relative humidity (RH), both in plastic and in steel surfaces. More recently, another study carried out in the human coronavirus 229E (Hu-CoV-229E) found that an inoculum of 10³ plaque forming units persisted on Teflon (PTFE), PVC, ceramic tiles, glass, and stainless steel for at least 5 days in an infectious state at 21 °C and a RH of 30% to 40%; whereas surfaces with copper and nickel rapidly inactivated the virus (Warnes et al., 2015). Lately, several surfaces were aerosolized with a COVID-19 tissue-culture infectious dose (van Doremalen et al., 2020). It was found that COVID-19 was more stable on plastic and stainless steel than on copper and cardboard, and viable viruses were detected up to 72 h after their application on these surfaces.

However, in these previous studies aerosols and/or inoculums containing coronaviruses were artificially generated to create an infectious environment. In our case, the infection of the surfaces could only be produced by patients’ aerosols as no-one could touch the surfaces and patients were isolated in their rooms. As the stability of several coronaviruses was previously tested in a number of surfaces, we decided to analyze the possibility of aerosol infection using 6 different surfaces. In our pilot study, the presence of COVID-19 was found in two different surfaces at 72 h in the room of a patient with a nasal cannula. Interestingly, no positives were found at 24 or 48 h in the same surfaces. It could be hypothesized that it is necessary an accumulation of virus in time to be detected using the RT-PCR technique. The rest of surfaces, placed in rooms with patients with no respiratory support, were not positive (Table 2).

These results lead us to two different hypotheses that should be clarified in future studies. The first of them is that glass and PP could be better surfaces for the detection of COVID-19. The second is that the use of respiratory support, such as nasal cannulas could promote the formation of aerosols allowing a higher movement of the air and consequently of the viruses, that could infect higher distances, so healthcare personnel should be more cautious in case of patients requiring ventilation support. In addition, our results, although preliminary, point the importance of coronavirus air transmission indoors and may shed some light in this debate.

4. Discussion

How SARS-CoV-2 virus is transmitted and the extent of environmental contamination is not clearly known (Ong et al., 2020). In recent studies, air transmission has been investigated in preliminary studies (Kenarkoohi et al., 2020; Ong et al., 2020). In one of them, 14 air samples in different wards of the indoor air of a hospital from Iran were analyzed using a liquid impinger biosampler, observing two positives from the ICU (Kenarkoohi et al., 2020). In another study, air samples and different surfaces from the rooms of 3 COVID-19 patients were investigated using SKC Universal pumps and sterile premoistened swabs, respectively. No positives were found in the air samples; however, swabs taken from the air exhaust outlets tested positive, suggesting that small virus-laden droplets might be displaced by airflows and deposited on equipment (Ong et al., 2020).

In previous studies, the exposition of aerosols or inoculums with coronavirus in several surfaces was performed, and estimation about their decay rates in time was studied (Duan et al., 2003; van Doremalen et al., 2013; Warnes et al., 2015). In one of the first studies analyzing the stability of SARS-CoV-1 in different surfaces, viruses were dropped onto the surfaces of the testing materials and maintained at room temperature (Duan et al., 2003). Higher infectivity was found in glass, metal and clothes, where they maintained their stability for more than 72 h. In 2013, during the outbreak of Middle East respiratory syndrome coronavirus (MERS-CoV), its stability in several surfaces was also tested under different environmental conditions (van Doremalen et al., 2013). MERS-CoV could still be recovered in its infectious form after 60 h at 20 °C and 40% relative humidity (RH), both in plastic and in steel surfaces. More recently, another study carried out in the human coronavirus 229E (Hu-CoV-229E) found that an inoculum of 10³ plaque forming units persisted on Teflon (PTFE), PVC, ceramic tiles, glass, and stainless steel for at least 5 days in an infectious state at 21 °C and a RH of 30% to 40%; whereas surfaces with copper and nickel rapidly inactivated the virus (Warnes et al., 2015). Lately, several surfaces were aerosolized with a COVID-19 tissue-culture infectious dose (van Doremalen et al., 2020). It was found that COVID-19 was more stable on plastic and stainless steel than on copper and cardboard, and viable viruses were detected up to 72 h after their application on these surfaces.

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4.1. Limitations, conclusions and future directions

This study has several limitations. Firstly, due to operational limitations during an outbreak, the number of patients, surfaces and times

| Step | Number of cycles | Temperature | Duration |
|------|------------------|-------------|----------|
| 1    | 1                | 50 °C       | 20 min   |
| 2    | 1                | 95 °C       | 15 min   |
| 3    | 1                | 94 °C       | 15 s     |
| 4    | 1                | 58 °C       | 30 s     |
| 5 (GO TO step 3) | 44             |             |          |
tested are relatively small, and larger studies are required to confirm our observations. Moreover, patients were selected randomly, being the only condition to have a positive RT-PCR test for SARS-CoV-2 virus. Thus, the authors of this study could not choose patients with higher viral load or patients with or without nasal cannulas. Additionally, the surfaces were placed in pairs and not all the surfaces could be included in all the patients’ rooms.

All these data support the recommendation to carry out frequent disinfection of the surfaces of not intubated, hospitalized patients, especially those with ventilation support, to avoid hand-mouth-nose and nosocomial infections in healthcare personnel. In addition, as this is a cheap and easy to perform method for COVID-19 detection, these COVID-19 traps could be used in public areas such as schoolrooms, courthouses, police offices, hospital waiting rooms, theatres or cinemas to rapidly detect a potential new outbreak of this mortal disease.

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**CRediT authorship contribution statement**

EOP conceived, coordinated and led all the study, performed sample collection and wrote the paper with the input from all the authors. FB designed the “COVID-19 traps” with the surfaces included for virus detection.
detection. DNC helped with the writing of the initial draft. AMD extracted RNA and performed all the RT-PCR experiments. JMM coordinated the handling of “COVID-19 traps” in the patients’ rooms. RM provided the surfaces and helped with their description. PR obtained financial support for the project.

**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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