Inanimate Objects and the Environment Are Far From Contributing to COVID-19 Spread

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Inanimate objects and the environment are far from contributing to COVID-19 spread

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Abstract

It is not clear if COVID-19 can be indirectly transmitted. It is not possible to conclude the role of environment in transmission of SARS-CoV-2 without studying areas in which people transit in great amounts, such as market areas. In this work we aimed to better understand the role of environment in the spread of COVID-19. We investigated the presence of SARS-CoV-2 in inanimate objects as well as in the air and in the sewage using RT-qPCR. We studied both, a reference market area and a COVID-19 reference hospital at Barreiras city, Brazil. We collected and analyzed a total of 268 samples from mask fronts, cell phones, paper moneys, card machines, sewage, air and bedding during the ascendant phase of the epidemiological curve of COVID-19 in Barreiras. As a result, we detected the human RNAse P gene in most of samples, which indicates the presence of human cells in specimens. However, we did not detect any trace of SARS-CoV-2 in all samples analyzed. To rule out the possibility of problems in sampling method we tested detection of SARS-CoV-2 by RT-qPCR in laboratory conditions to reproduce environmental temperature and humidity. As a result, we showed detection of the virus in different conditions. We conclude that our sampling method reliable and that, strikingly, the environment and inanimate materials do not have an important role in COVID-19 transmission.

Keywords: COVID-19, environment, spread, indirect transmission, RT-qPCR

Introduction

COVID-19 (coronavirus disease 2019) is caused by the new coronavirus SARS-CoV-2 (*Severe acute respiratory syndrome coronavirus 2*) identified in China, in late 2019 [1–3]. The disease is highly efficiently transmitted and was spread to all continents, becoming a pandemic. According to the World Health Organization (WHO), more than 109,217,366 cases with more than 2,413,912 deaths were confirmed worldwide as of 17 February 2021 [4,5]. To date, there is no specific antiviral drug capable of efficiently controlling SARS-CoV-2, although therapies based on anti-coagulants and convalescent plasma have been shown to be promising [6–8]. Fortunately, there are several vaccine formulations approved for use in humans [9]. However, vaccination of the majority of populations is not being achieved, which prevents the containment of the pandemic. In addition, the raising of genetic variants of SARS-CoV-2 with increased transmission capacity and immune escape potential is a relevant concern [10], due to the possibility of both, increasing in transmission speed of COVID-19 and compromising of vaccines efficacy.

The cost of dealing with the COVID-19 is huge. Hospitalization, testing, tracing, mitigating strategies, masks and cleaning of inanimate materials demand a new routine of increased costs for governments and businessmen and also impact the domestic budgets. The increased use of disinfectants in inanimate materials is now a new normal scenario, due to the concern regarding indirect transmission of SARS-CoV-2. By the end of 2020, a total of US$4.5 billion in disinfectants was sold, an increase of more than 30% over 2019 [11]. However, it is not clear if COVID-19 can be indirectly transmitted.

The presence of SARS-CoV-2 in the air and inanimate objects of intensive care units was reported in the middle of 2020 and beggening of 2021 [12–14]. However, the viral load was not informed. It is not possible to conclude the role of environment in transmission of SARS-CoV-2 without sampling of areas in which people transit in great amounts, such as market areas. Are we exaggerating the risk of transmission of COVID-19 by fomites? What is the risk of SARS-CoV-2 transmission by fomites in real-life conditions? In this work we aimed to answer these questions and contribute to the understanding regarding the role of environment in the spread of COVID-19.

Materials and methods

Aim and specific aims

In this study, we aimed to understand the role of the environment in the spread of COVID-19. Specific aims were: i) to investigate the presence of SARS-CoV-2 in inanimate objects using RT-qPCR and ii) to investigate the presence of SARS-CoV-2 in the air and in the sewage using RT-qPCR.

Study area

The investigation was carried out in the city of Barreiras, located in the western region of the state of Bahia, Brazil. It is the 10th largest city in the state and the largest in the west of Bahia, with more than 150 thousand inhabitants [15]. Due to its economic potential and health structure, the city receives people from all over the western region and now, in the pandemic period, it receives a relevant number of COVID-19 patients, as Barreiras has the largest number of beds in Intensive Care Units (ICU)[16] in the west of Bahia.

Study design

In order to verify the presence of SARS-CoV-2 in the environment, we studied the main market area of Barreiras which includes stores, supermarkets, restaurants, snack bars, bars and a variety of commercial points. We also studied the Eurico Dutra Hospital, a city reference health unit for COVID-19. We collected samples of mask fronts, cell phones, paper moneys, card machines, sewage, air and bedding. The study was conducted during the ascendant phase of the epidemiological curve of COVID-19 in Barreiras. Viral detection using the RT-qPCR method was performed at the Laboratório de Agentes Infecciosos e Vetores, Universidade Federal do Oeste da Bahia.

Environmental samples

Sampling was carried out from June 1 to August 3, 2020. Three of these samplings were carried out at the main market area of Barreiras (an open public place with intense circulation of people). On June 1 (maximum temperature of 31.3°C; minimum humidity of 49%) we collected five samples of sewage, 27 samples from cell phones and 30 samples from paper money. On June 12 (maximum temperature of 32.3°C; minimum humidity of 33%) we collected nine samples of air, 36 samples from mask fronts, 30 samples from paper money, 10 samples from card machines and 12 samples from cell phones. On June 26 (maximum temperature of 31.6°C; minimum humidity of 40%), at the Eurico Dutra Hospital, we collected 12 samples of air from wards that admit patients with COVID-19, as well as 27 samples of masks (including health professionals and COVID-19 patientes) and 12 samples from bed linen of patients admitted with COVID-19 confirmed by RT-qPCR. We also collected samples from 12 cell phones owned by patients and health workers. On August 3 (maximum temperature of 30.3°C; minimum humidity of 30%), again at the market area, we collected 12 samples from mask fronts, 12 samples from card machines, 30 samples from paper money and 18 samples from cell phones. In total, we collected 268 samples from the environment during the ascendant phase of the epidemiological curve of COVID-19 in Barreiras.

For the collection of inanimate objects, the following materials were used: 15 mL tubes with 2 mL of sterile saline solution [NaCl 0.9% (v/v)] and sterile swabs. The saline-soaked swabs were rubbed on surfaces of the materials. After collection, they returned to their respective identified tubes. Sterile Pasteur pipettes were used to collect 2 mL of sewage samples in each of five different points of the market area. Air samples were collected by retention of particles using a high volume sampler (Energética, Brazil) with polytetrafluoroethylene (PTFE) filter and a regenerated cellulose filter, each 47 mm in diameter and 0.22 μm pores. The instrument was left on for 1 hour per collection and membranes were removed and placed in sterile 15 mL tubes containing 2 mL of sterile saline. Tubes containing membranes were vortexed in order to suspend air particles in sterile saline. After each collection, the samples were stored at 8 °C and sent for viral RNA extraction within six hours.

Epidemiological data

Epidemiological data about the COVID-19 pandemic scenario in Barreiras were taken from the epidemiological bulletins on the website of the city's municipal health department [17]. The numbers of total cases, active cases and deaths were used in the study and collected from the date of the first case notified in Barreiras (March 21, 2020) and completed at the time of the study closure (August 19, 2020). The newsletter comes with the following information: notified cases, positive cases, discarded cases and awaiting results. Within the number of positive cases, there is a subdivision showing the number of recovered cases; in home isolation; hospitalized and deaths. To calculate the number of active cases, it was necessary to subtract the number of positive cases by the number of recovered and deaths.

RNA extraction and RT-qPCR

The nucleic acid extraction from environmental samples was performed using the PureLink® Viral RNA / DNA Mini Kit, following the manufacturer's protocol.

RT-qPCR assays were carried out using 2.5 µL of purified RNA, 4.5 µL of ultrapure H_2O, 2.5 µL of TaqMan™ Fast Virus 1-Step Master Mix (Applied Biosystems) and 0.75 µL of primers and probes (Integrated DNA Technologies - IDT primers and probes for N1, N2 or RP assays, in CDC's recommended working concentrations), in a final volume of 10 µL reaction. Thermocycling was carried out in a QuantStudio 5 instrument (Applied Biosystems) with a hold stage composed of a first step of 5 min at 50 °C, followed by a second step of 20 s at 95 °C. The PCR stage was composed of a first step of 15 s at 95 °C followed by a second step of 1 min at 55 °C, repeated 45 times. Cycle thresholds (CT) values ≤ 34.1 were interpreted as positive, between 34.1 and 35 as inconclusive and from 35.1 to 45 as negative for SARS-CoV-2, according to our internal standard curve, cut off and decision matrix constructed based on assays carried out with a control plasmid harboring the N gene (2019-nCoV_N_Positive Control – IDT). CT values of RT-qPCR and our standard curve were used as indicators of the copy number of SARS-CoV-2 genome copies (GC) in specimens with lower cycle threshold values corresponding to higher viral copy numbers, as previously described [18].

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195

196 Testing reliability of sampling method

197 The virus used in this experiment is a SARS-CoV-2 and was stored in the
198 Laboratório de Agentes Infecciosos e Vetores of the Universidade Federal do Oeste da
199 Bahia as part of the routine molecular diagnosis of COVID-19 based on RT-qPCR. We
200 tested two materials: glass and fabric; in two different conditions: outdoors and indoors.
201 Temperature and humidity were measured in all conditions during the experiment and
202 are presented in Table 1. Sterile materials (glass or fabric) were contaminated in
203 different areas of their surfaces with an equivalent to 500,000 genome copies in 50 µL
204 of SARS-CoV-2 suspension in each area. The contamination was carried out into a
205 biological safety cabinet class II-B2 and left to air dry for 20 min. The materials were
206 incubated into the biological safety (indoors) or at a sealed box exposed to
207 environmental conditions (outdoors). Samples were collected with a saline-soaked
208 swab, as described above and submitted to RNA extraction and RT-qPCR. Samples
209 were collected immediately after contamination and air dry of materials (point 0) and on
210 times 1.5 h, 3h, 6h and 12h.

211

212 Statistical analysis

213 We performed statistical analysis using PRISM version 5.1. We compared
214 categorical variables using Analysis of Variance test (ANOVA) with Bonferroni's
215 multiple comparison test. A p-value ≤ 0.05 was considered as statistically significant.

216

217 **Results**

218 Epidemiological surveillance

219 As shown in Figure 1A, we carried out our first sampling in the beginning of the
220 ascendance of COVID-19 curve in Barreiras city, on June 1, 2020. The last sampling
221 was carried out with around 1,000 active notified cases in the city. By the end of
222 sampling 25 deaths had been counted due to COVID-19 (Figure 1B). Collectively, these

results indicate that our sampling was carried out during the active circulation of SARS-CoV-2 in Barreiras city.

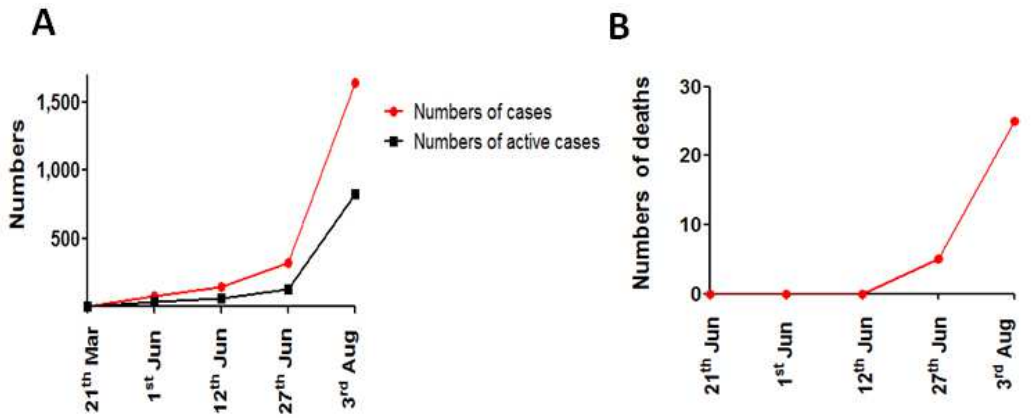

Figure 01. COVID-19 pandemic scenario in the city of Barreiras, Brazil, during the study. A, numbers of cases of COVID-19 (total and active cases). The counting of COVID-19 cases was initiated in March 21, 2020, with the first confirmed case. Samplings were carried out on June 1, 12 Junes, June 27 and August 3, 2020. B, numbers of deaths. The first death due to COVID-19 was reported in June 13, 2020.

Lack of detection of SARS-CoV-2 in the environment during ascendant curve of COVID-19 in Barreiras city

As shown in Table 2, the human RNAse P gene was detected in almost all samples, showing the presence of human cells in most of specimens. However, SARS-CoV-2 was not detected in any kind of sample collected in both, the market area and the COVID-19 reference hospital. This is a striking result that indicates that the virus was not present in the environment or inanimate objects during the ascendant curve of COVID-19 cases in Barreiras city.

Proof of reliability of sampling method

In order to rule out that the sampling method was not suitable to detect SARS-CoV-2 in environment and inanimate objects using RT-qPCR we designed an experiment to verify if using saline-soaked swabs to collect fonts of nucleic acids is reliable. In addition, we aimed to verify if high temperatures and low humidity typical of Barreiras

city was interfering in detection capacity. As shown in Figure 2, SARS-CoV-2 RNA could be detected in both, glass and fabric, independently of conduction of the experiment in laboratory or outside conditions. Even with visible differences in temperatures and humidity in the two different conditions the detection of viral RNA by RT-qPCR was not prevented. The cycle threshold was more relevantly increased in fabric than in glass. In addition, the detection was significantly more sensible in glass (see Figure 2). These results clearly show that the saline-soaked swab collects fonts of nucleic acids. Moreover, the sampling and detection of viral RNA are not affected by temperature or humidity. Collectively, these results indicate that our sampling method is reliable.

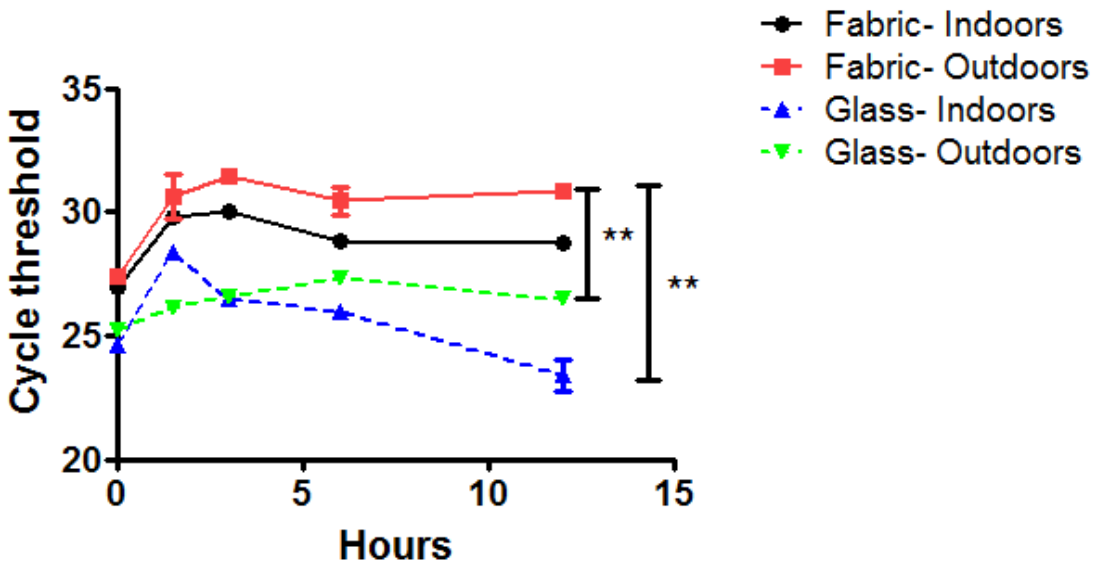

Figure 2. Cycle thresholds (CT) for detection of SARS-CoV-2 in the different contaminated materials in different conditions and time points. Analysis of variance with Bonferroni's multiple comparison test. Statistical significance was set as p≤ 0.05. **, p ≤ 0.05.

Environment and indirect transmission of SARS-CoV-2

Indirect transmission depends on the presence of a given infectious agent in inanimate objects and/or the environment. Our results show that SARS-CoV-2 was not present in inanimate objects or environmental samples during the ascendant curve of COVID-19 cases in Barreiras city, Brazil. We also showed that our sampling method is reliable. As proposed in Figure 3, together, these results indicate that inanimate objects

and the environment were far from contributing to COVID-19 spread. The person-to-person transmission seems to have a central role in COVID-19 spread.

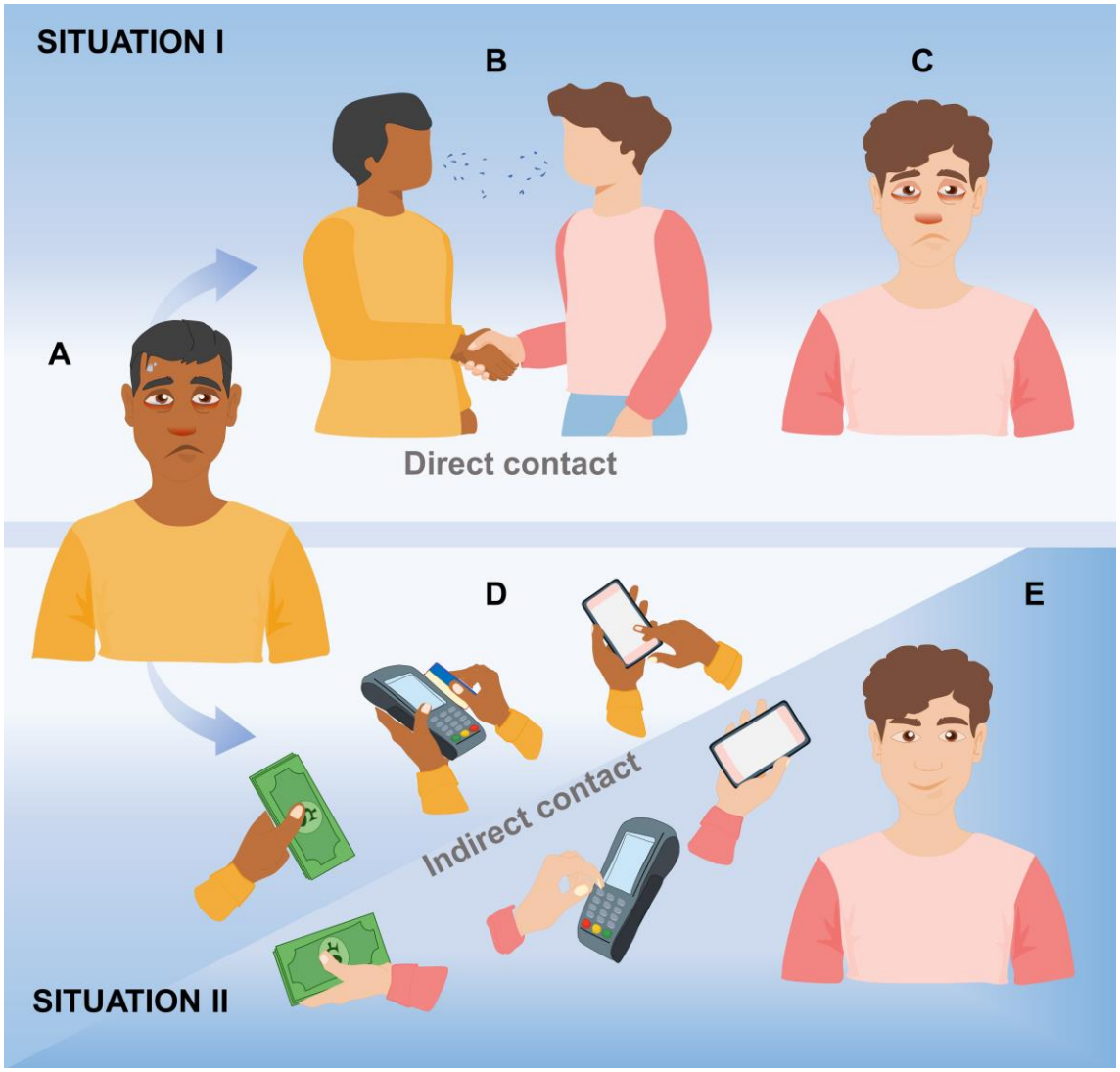

Figure 3. Direct contact as the main form of transmission of SARS-CoV-2. A, man with COVID-19. B, an infected man comes into direct contact with a healthy person and talks, neglecting the use of a mask and social distance. C, after contact with the infected person, the second man becomes infected with viruses. D, an infected man comes into contact with different inanimate objects. E, a healthy man comes into contact with inanimate objects that have been manipulated by an infected person and is not infected.

Discussion

In this work we aimed to contribute to the understanding of the role of environment in the spread of COVID-19. The literature contains reports showing detection of SARS-CoV-2 in hospital environments [12,13,19]. However, it has been recently reported that the risk of acquiring COVID-19 by touching contaminated surfaces in less than 5 in 10,000 [20]. In addition, although SARS-CoV-2 RNA has been detected in hospital environment the virus could not be isolated in cell culture [19]. The old school shows that respiratory diseases caused by viruses such as Rhinovirus colds are not indirectly transmitted [21]. So, do we need to keep investing billions of dollars in disinfecting environments and inanimate objects in the COVID-19 pandemic?

Our results show that SARS-CoV-2 is not detectable in the environment or in inanimate objects during its active circulation if human hosts. The detection of human RNAse P gene in samples indicates that human cells of their fragments were present in studied materials. We stress that we did not detect any trace of SARS-CoV-2 in samples studied by us. We analyzed more than 250 samples collected in different occasions during the ascendant curve of COVID-19 cases in Barreiras city. These samples were collected from a market area with intense circulation of people and also from a reference health unit for COVID-19. In addition to the lack of SARS-CoV-2 in samples form cell phones, card machines, mask fronts and paper money from the market area we did not detect viral RNA even from bedding and front masks of hospitalized COVID-19 patients. Although we have these striking results, one could argue that our sampling method was not recovering enough fonts of nucleic acid for molecular detection by RT-qPCR.

To rule out that viral detection has been affected by the sampling method we designed an experiment to verify it. Although we have already shown that human RNAse P gene could be detected, the recovery of viral RNA in environmental or inanimate objects could be difficult. The amplification of RNAse P gene can be provided by using DNA as a template. However, amplification of SARS-CoV-2 targets can be provided only using RNA as a template in this situation. Our results indicate that the virus can be detected by RT-qPCR independently of time of exposure to environment until 12h. In addition, detection is not prevented by variations in temperature or humidity. We could argue that the hostile clime of Barreiras with

elevated temperatures and low percentages of humidity could be preventing molecular detection of SARS-CoV-2 in this study. But our results show that even with a temperature of 43.8°C for 6h of exposure at outdoors conditions the viral RNA could be detected. We found only a difference in cycle thresholds for recovery from glass or fabric. The amplification of SARS-CoV-2 targets in samples recovered from fabric was significantly lower than those from glass. We hypothesize that adsorption of viral particles and/or viral RNA by fabric decrease the recovery of amplifiable RNA. Thus, detection of coronavirus RNA in bedding and front masks from COVID-19 patients can be limited or prevented by the characteristics of the fabric.

Here, we did not studied the same kind of inanimate objects previously reported as font of SARS-CoV-2 RNA [12,13]. We investigated objects of high frequent contact with hands in the market area, such as card machines, paper money and cell phones. In the hospital environment we studied objects that we really expected to detect SARS-CoV-2 RNA, such as bedding and front masks of COVID-19 patients. But for our surprise, we did not detect any trace of viral RNA. These differences in results found in our studies can indicate a limitation in the concentration of search for coronavirus in hospital environments. The disease is not mainly transmitted in hospitals, but in daily locations such as restaurants, stores, schools, at home, at work and in bars and pubs.

Our results indicate that inanimate objects and the environment are far from contributing to COVID-19 spread or, at least, that investigations regarding indirect transmission should consider studying daily locations in which people coexist. We stress that physical distancing, wearing masks and other non-pharmacological interventions used to control other respiratory viral diseases are essential to control COVID-19 until we have the majority of populations vaccinated. However, it seems we are exaggerating in using tons of disinfectants in inanimate objects. The person-to-person transmission is more likely to have a central role in COVID-19 spread.

Declarations

Availability of data and materials

Data will be provided under request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Author's contributions: ALSR and JRP carried out sampling, RT-qPCR and analyses, interpreted the data generated, prepared figures and wrote the manuscript; TCN and JDSS interpreted the data generated and wrote the manuscript; BR interpreted the data generated, prepared figures and wrote the manuscript; RCK interpreted the data generated and wrote the paper; AB interpreted the data generated and wrote the manuscript; JHA conceived the study, collected the samples, interpreted the data generated and wrote the manuscript MVFC collected the samples and carried out RT-qPCR; CP prepared figures, interpreted the data and wrote the paper; AB. All authors read and approved the final version of the manuscript.

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Table 1. Temperature and humidity in conditions of the experiment carried out to evaluate the sampling method.

	External environment		Internal environment	
Time (hours)	Temperature °C	Humidity %	Temperature °C	Humidity %
0	24.3°C	50%	24.3°C	50%
1.5	32.5°C	47%	21°C	64%
3	34.4°C	43%	21.4°C	65%
6	43.8°C	24%	20.8°C	74%
12	22.9°C	81%	21.4°C	70%

Table 2. Mean and standard deviation of Cycle thresholds (CT) of the RNAse P gene detected by RT-qPCR in samples collected.

	Samples						
	Mask	Cell Phone	Paper money	Sewage	Air	Bedding	Card machine
Mean	36,592	35,140	34,138	37,004	0	0	32,753
STD*	3,746	2,488	2,288	3,430	0	0	7,440

[a] STD= standard deviation.

Figure legends

Figure 1. COVID-19 pandemic scenario in the city of Barreiras, Brazil, during the study. A, numbers of cases of COVID-19 (total and active cases). The counting of COVID-19 cases was initiated in March 21, 2020, with the first confirmed case. Samplings were carried out on June 1, 12 Junes, June 27 and August 3, 2020. B, numbers of deaths. The first death due to COVID-19 was reported in June 13, 2020.

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Figures

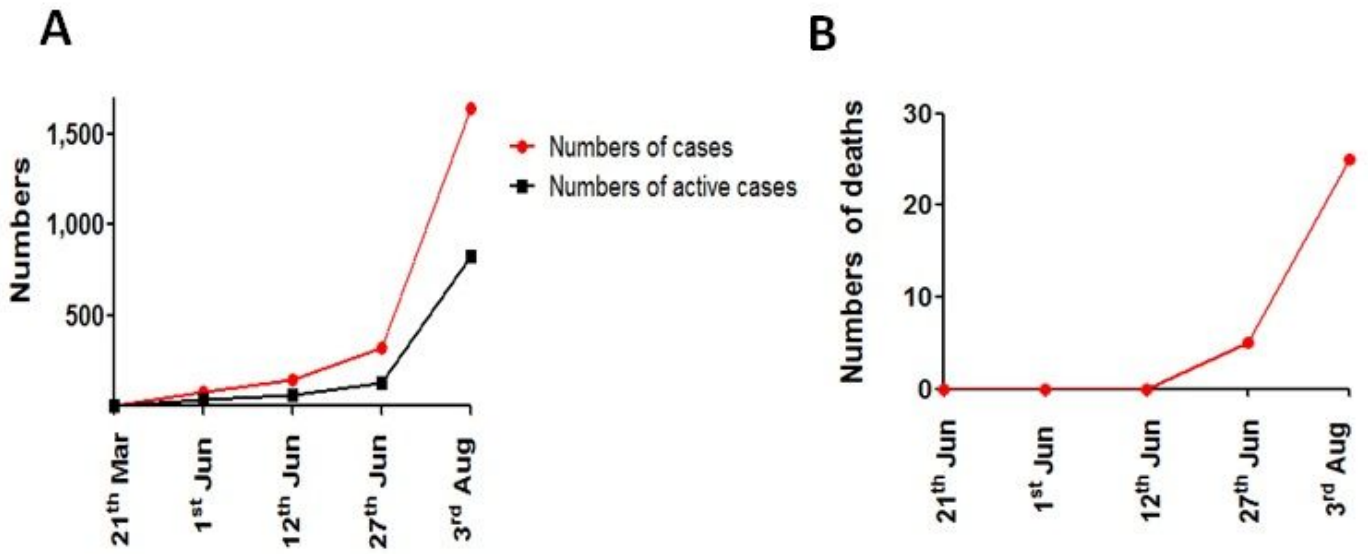

Figure 1

COVID-19 pandemic scenario in the city of Barreiras, Brazil, during the study. A, numbers of cases of COVID-19 (total and active cases). The counting of COVID-19 cases was initiated in March 21, 2020, with the first confirmed case. Samplings were carried out on June 1, 12 Junes, June 27 and August 3, 2020. B, numbers of deaths. The first death due to COVID-19 was reported in June 13, 2020.

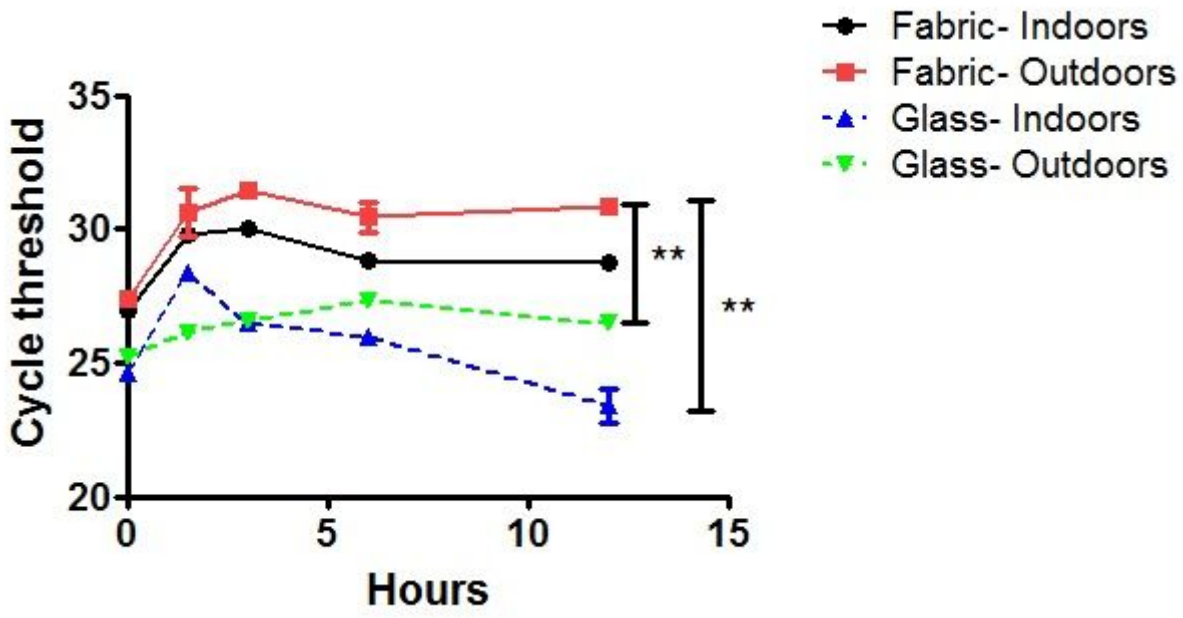

Figure 2

Cycle thresholds (CT) for detection of SARS-CoV-2 in the different contaminated materials in different conditions and time points. Analysis of variance with Bonferroni's multiple comparison test. Statistical significance was set as $p \leq 0.05$. **, $p \leq 0.05$.

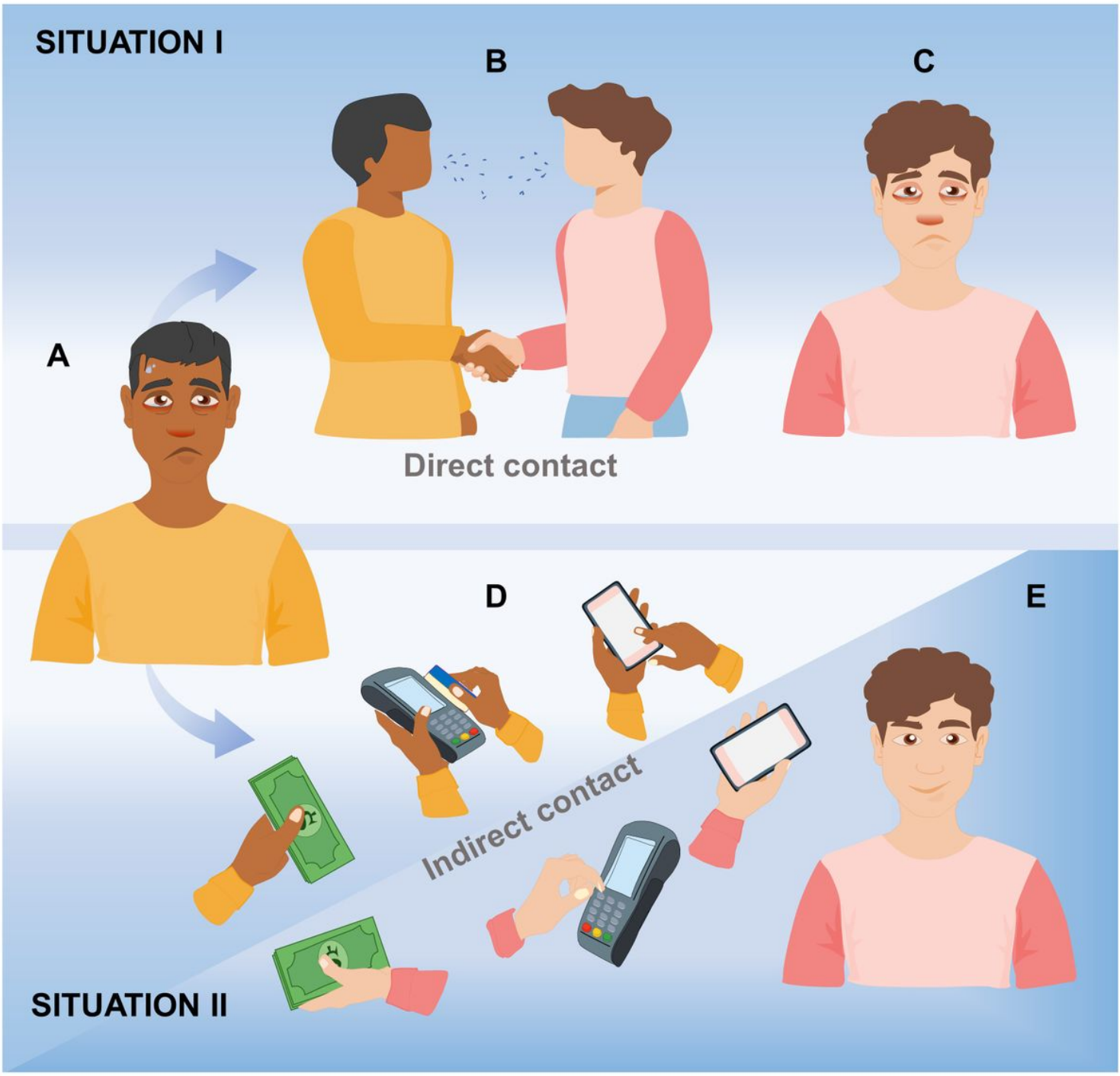

Figure 3

Direct contact as the main form of transmission of SARS-CoV-2. A, man with COVID-19. B, an infected man comes into direct contact with a healthy person and talks, neglecting the use of a mask and social distance. C, after contact with the infected person, the second man becomes infected with viruses. D, an

infected man comes into contact with different inanimate objects. E, a healthy man comes into contact with inanimate objects that have been manipulated by an infected person and is not infected.