Widespread Contamination of SARS-CoV-2 on Highly Touched Surfaces in Brazil During the Second Wave of the COVID-19 Pandemic

Severino Jefferson Ribeiro da Silva[1], Jéssica Catarine Frutuoso do Nascimento[1], Wendell Palôma Maria dos Santos Reis[1], Caroline Targino Alves da Silva[1], Poliana Gomes da Silva[1], Renata Pessôa Germano Mendes[1], Allyson Andrade Mendonça[1], Bárbara Nazly Rodrigues Santos[1], Jurandy Júnior Ferraz de Magalhães[1,2,3], Alain Kohl[4] and Lindomar Pena[1*]

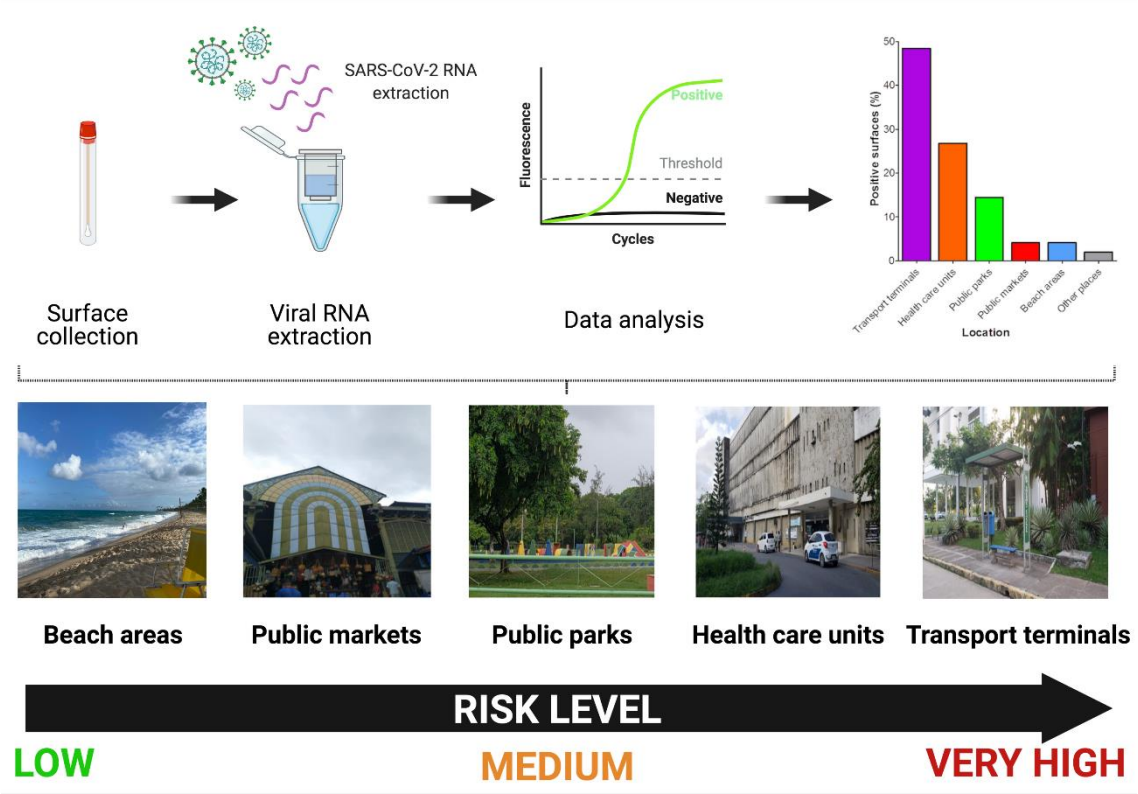
[1]Laboratory of Virology and Experimental Therapy (LAVITE), Department of Virology, Aggeu Magalhães Institute (IAM), Oswaldo Cruz Foundation (Fiocruz), 50670-420, Recife, Pernambuco, Brazil.

[2]Department of Virology, Pernambuco State Central Laboratory (LACEN/PE), Recife, Pernambuco, Brazil.

[3]University of Pernambuco (UPE), Serra Talhada Campus, Serra Talhada, Pernambuco, Brazil.

[4]MRC-University of Glasgow Centre for Virus Research, Glasgow, G61 1QH, UK.

*Corresponding author: lindomar.pena@fiocuz.br



ABSTRACT

Although SARS-CoV-2 surface contamination has been investigated in temperate climates, few studies have been conducted in the tropics. Here, we investigated the presence of SARS-CoV-2 on high-touch surfaces in a large city in Brazil. A total of 400 surface samples were collected in February 2021 in the City of Recife, Northeastern Brazil. A total of 97 samples (24.2%) tested positive for SARS-CoV-2 by RT-qPCR using the CDC-USA protocol. All the collection sites, except one (18/19, 94.7%) had at least one environmental surface sample contaminated. SARS-CoV-2 positivity was higher in public transport terminals (47/97, 48.4%), followed by health care units (26/97, 26.8%), public parks (14/97, 14.4%), public markets (4/97, 4.1%), and beach areas (4/97, 4.1%). Toilets, ATMs, handrails, playground, and outdoor gym were identified as fomites with the highest rates of viral contamination. Regarding the type of

material, SARS-CoV-2 RNA was found more commonly on metal (45/97, 46.3%), followed by plastic (18/97, 18.5%), wood (12/97, 12.3%), rock (10/97, 10.3%), concrete (8/97, 8.2%), and glass (2/97, 2.0%). Taken together, our data indicated extensive SARS-CoV-2 contamination in public surfaces and identified critical control points that need to be targeted to break SARS-CoV-2 transmission chains.

Keywords: SARS-CoV-2; Coronavirus disease 2019; Environmental contamination; Prevention policies; Transmission.

Synopsis

We investigated the presence of SARS-CoV-2 on high-touch surfaces in a large city in Brazil and identified critical points to establish effective control measures aimed at breaking transmission.

INTRODUCTION

Coronaviruses (CoVs) are members of the *Coronaviridae* family and represent a diverse group of viruses that cause respiratory and intestinal infections in animals and humans [1]. The *Coronavirinae* subfamily is divided into four genera - *Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus*. Alphacoronaviruses (HCoV-229E and HCoV-NL63) and Betacoronaviruses (HCoV-OC43 and HCoV-HKU1) are commonly associated with mild respiratory disease in humans [2]. However, in the last two decades, three highly pathogenic betacoronaviruses have emerged from animal sources to cause severe respiratory disease in humans: severe acute respiratory syndrome coronavirus (SARS-CoV) [3], Middle East respiratory syndrome coronavirus (MERS-CoV) [4], and more recently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[5-7].

SARS-CoV-2 first emerged in the city of Wuhan, Hubei province, China, in December 2019 causing an outbreak of a yet unknown acute pneumonia [8]. Unlike SARS-CoV and MERS-CoV, the new coronavirus was found to be highly transmissible among humans and has spread rapidly around the globe prompting the World Health Organization (WHO) to declare a pandemic on March 11, 2020 (WHO, 2020). As of June 7, 2021, there have been approximately 173.4 million confirmed cases of COVID-19 across the world, with over 3.7 million deaths [9]. Difficult to control viral transmission allied with the slow progress in the rollout of COVID-19 vaccines in most countries have contributed to the emergence of new variants of concern of SARS-CoV-2, which are more transmissible and can escape from natural and vaccine-acquired immunity [10-13].

SARS-CoV-2 is spread person to person mainly through exposure to respiratory fluids containing infectious virus. Virus exposure can occur in three main ways, which are not mutually exclusive: (i) inhalation of infectious virus present in very small fine droplets and aerosol particles; (ii) deposition of virus on exposed mucous membranes in the mouth, nose, or eye by direct splashes and sprays, and (iii) touching mucous membranes with hands contaminated by exhaled respiratory fluids containing virus or from touching fomites containing the virus[14]. Notably, SARS-CoV-2 has been found to have high person-to-person transmission through direct contact with infected individuals [15], especially by coughing, sneezing and even breathing/ talking by an infected person [16-19]. SARS-CoV-2 enters the body through the mucous membranes of the eyes, mouth or nose and spreads to the nose line, sinus cavity, and throat until deposition into the human respiratory tract [20]. Although transmission through direct contact, or airborne (respiratory droplets and/or aerosols) are considered to be the dominant routes for the spread of COVID-19[21, 22], the transmission dynamics of SARS-CoV-2 by environmental surfaces and their role in the transmission chain remain unclear, and probably multifactorial. The risk of infection is influenced by the distance from the source, the amount of virus to which a person is exposed and the length of time since the virus has been deposited on the surface, since SARS-CoV-2 viability over time is influenced by environmental factors such as type of surfaces, temperature, humidity, and ultraviolet radiation (e.g., sunlight) [21, 23, 24].

Thus, understanding of distribution and patterns of environmental contamination by SARS-CoV-2 are relevant information for public health

114 authorities. This knowledge allows the identification of critical points to
115 establish effective control measures aimed at breaking transmission.

116 Several recent studies have investigated the presence of SARS-CoV-2
117 RNA in air and environmental surfaces, especially in health care settings [25-33].
118 Previous studies under controlled laboratory conditions have demonstrated the
119 ability of SARS-CoV-2 to remain infectious on different types of common
120 surfaces, such as stainless steel, glass and paper, for up to 28 days at 20 °C [34],
121 and it can also remain infectious in aerosols for up to 3 h [35]. However, little is
122 known about SARS-CoV-2 contamination of environmental surfaces in tropical
123 public areas with a large flow and concentration of people. Therefore, studies
124 investigating the presence of SARS-CoV-2 RNA on surfaces, and the infectious
125 potential of these particles are of paramount importance.

126 To address this gap of knowledge, we investigated the presence of
127 SARS-CoV-2 RNA on highly touched surfaces in Recife, a large city in
128 Pernambuco state with a tropical monsoon climate. Samples were collected
129 during the second wave of the COVID-19 in Brazil, one of the most severely
130 affected countries by the pandemic. Our findings showed widespread viral
131 contamination across many urban public settings and poor adherence to
132 COVID-19 mitigation measures. Our data provide a real-world picture of SARS-
133 CoV-2 dispersion in highly populated tropical areas and identify critical control
134 points that need to be targeted to halt SARS-CoV-2 transmission.

135

136

137

MATERIAL AND METHODS

Study design and setting

This study was conducted in Recife, the capital of Pernambuco state, which is one of the most densely populated metropolitan regions in Brazil with 1,537,704 million people (https://cidades.ibge.gov.br/brasil/pe/recife). The city is located on the coast of Northeast coast of Brazil and has a tropical monsoon climate under the Köppen climate classification, with warm to hot temperatures and high relative humidity throughout the year.

This prospective cross-sectional study was designed busy areas and with a large flow and concentration of people. Initially, we subdivided Recife's highly frequented places into 6 categories, including: a) transport terminals; b) health care units; c) public parks; d) public markets; e) beach areas; f) other public places (food supply center). A total of 400 environmental surface specimens were collected between Feb 2 and Feb 25, 2021 (Figure 1). Samples were collected between 9:00 a.m. and 1:00 p.m. During sample collection, the temperature was between 26°C to 32°C (average temperature 29°C) and the average humidity was 72%. Environment data was obtained from Time and Date AS website (http://www.timeanddate.com/weather/brazil/recife/climate). This coincided with a period of progressive increase in the number of COVID-19 cases in Pernambuco state and Brazil, representing the second wave of the COVID-19 pandemic in this part of the world (Figure 2) and also the beginning of COVID-19 vaccination efforts in this state. The ongoing pandemic of COVID-19 in the Pernambuco state has resulted in 499,572 laboratory-confirmed cases and 16,292 deaths as of 6 June 2021[36]. It is important to highlight that Recife

has a high concentration of specialized hospitals and is considered a reference health center for the Northeast region of Brazil.

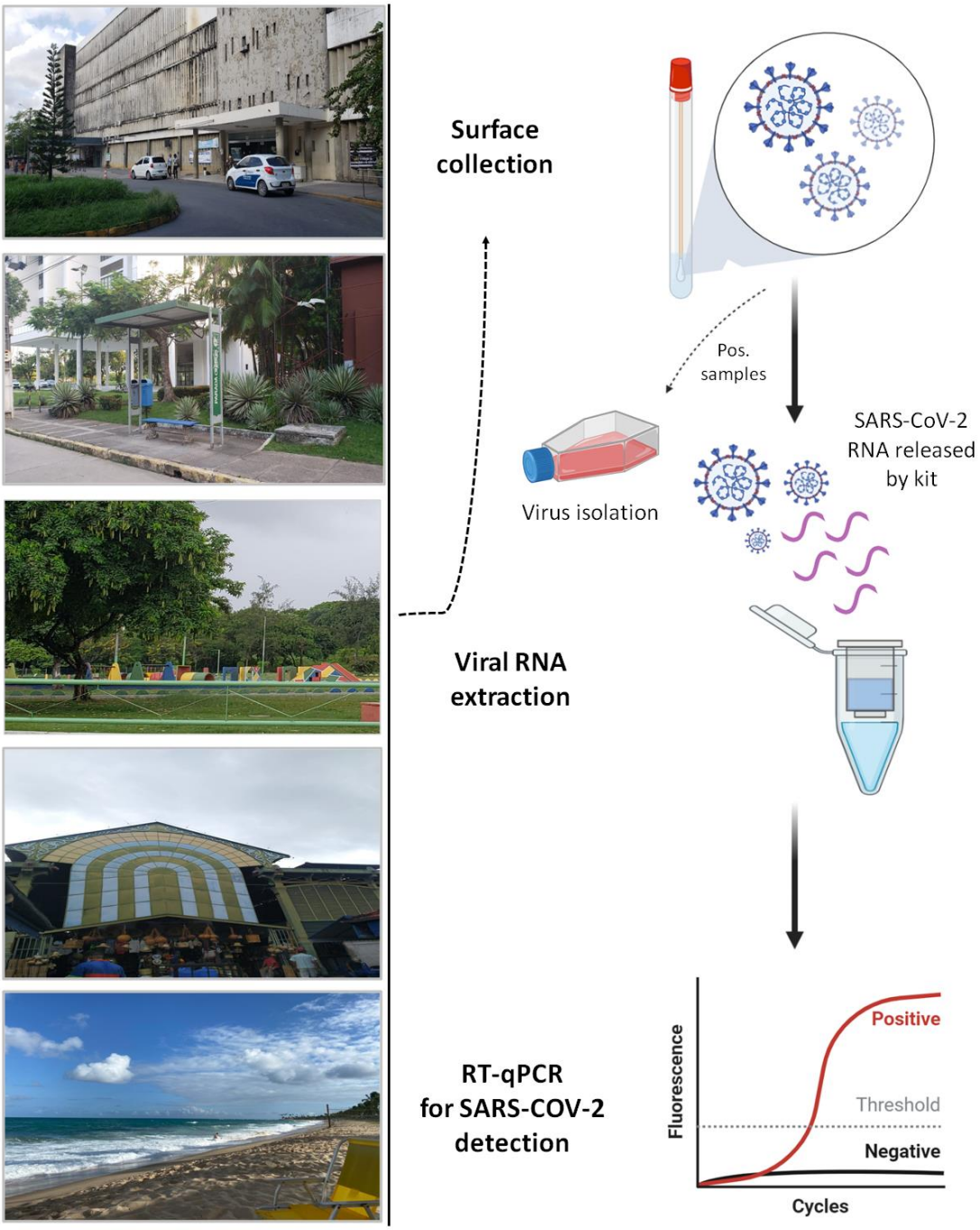


Figure 1. Study design showing the collection points of surface samples and the graphical workflow used to test the swabs. Created with Biorender.com

Sampling areas

Transport terminals

A total of 84 surface samples were collected from four public transport terminals with a large daily passenger flow and concentration. We strategically selected transport terminals that connect several cities in the metropolitan region of Recife. Twenty-one swabs were collected for each transport terminal. The collection points included the external area of the transport terminal and neighboring areas: (1) bus terminal entrance; (2) bus terminal exit; (3) bus terminal access; (4) subway station access; (5) ATM; (6) toilet; (7) handrail; (8) bench; (9) bus stop; (10) counter; (11) faucet; (12) ticket machine.

Health care units

A total of 84 surface samples were collected from four reference hospitals for treatment of COVID-19 patients in Recife, Brazil. Twenty-one swabs were collected for each hospital. The collection points included the external area of the hospital and neighboring areas: (1) principal entrance; (2) hospital access; (3) ambulatory entrance; (4) patient sample collection area; (5) toilet; (6) traffic light button; (7) coffee shop; (8) public phone; (9) bus stop; (10) resting area.

Public parks

A total of 105 surface samples were collected from five public parks. We strategically selected parks with high visitor flow, including children who access the playground. Twenty-one swabs were collected for each public park. The collection points included: (1) playground; (2) recreation area; (3) outdoor gym;

(4) toilet; (5) handrail; (6) bus stop; (7) public bike station; (8) traffic light button; (9) coffee shop; (10) faucet.

Public markets

A total of 85 surface samples were collected from four public markets. Twenty-one swabs were collected for each public market with exception of one, where we collected twenty-two swabs. The collection points included: (1) principal entrance; (2) side entrance; (3) public market access; (4) toilet; (5) kiosk; (6) store; (7) food hall; (8) traffic light button; (9) faucet; (10) resting area; (11) outside area.

Beach areas

A total of 21 surface samples were collected from two beaches located in the coastal area of Recife, Brazil. Interestingly, the visited beaches had a high concentration of people during the time of surface collection and during all times of restrictive relaxation measures established by the state government during the COVID-19 pandemic. The collection points included: (1) toilets; (2) benches; (3) public bike station; (4) outdoor gym; (5) fresh green coconut; (6) handrails; (7) faucet; (8) traffic light button; (9) bus stop; (10) resting area.

Other areas

A total of 21 surface samples were collected from one food distribution center located in Recife, Brazil. We selected this place as it is a place which serves as a gateway for people from all over the Brazilian territory, and acts as a source of food supply for the Northeast of Brazil. The collection points included: (1) toilet; (2) restaurant; (3) handrail; (4) resting area.

Surface sampling

Environmental samples were collected by qualified technicians who had received biosafety training and were equipped with personal protective equipment. For sample collection, sterile swabs (bioBoa Vista, Brazil) were used, that were put into a conical tube (15 mL) containing 2 mL of virus preservation solution (sterile phosphate-buffered saline, pH 7.2). Each swab was vigorously rubbed on the surface with a collection area of 25 cm^2. Samples were collected from distinct types of materials, including metal, plastic, wood, rock, concrete, and glass. The time of collection and climate conditions of the day were recorded during sampling. In addition, an environmental site assessment questionnaire was applied to identify whether the collection environment and the population were following public health measures for preventing the rapid spread of SARS-CoV-2 and, subsequently, the COVID-19 transmission.

Sample transfer and processing

Surface samples were collected and immediately stored at 4 °C prior to transfer to the biosafety level 3 laboratory (BSL-3) of Fiocruz Pernambuco, Brazil, where all samples were processed until 72 h after collection. After processing, each sample was taken directly tested according to the instructions described below.

Viral RNA extraction and RT-qPCR for SARS-CoV-2 detection

Viral RNA was extracted from surface samples (140 µL of transport solution) using the QIAamp Viral RNA Mini Kit (QIAGEN, Germany) following the manufacturer's protocol. RT-qPCR assay targeting the N protein according to protocols recommended by the Centers for Disease Control and Prevention - CDC USA, was used to detect SARS-CoV-2 (Supplementary Table 1) [37]. Samples were considered positive when they presented amplification for N1 target, considering the threshold for cycle quantification (Cq) value of 40 [37]. Samples with Cq ≥ 40 were considered as negative. Briefly, each reaction was prepared using the QuantiNova Probe RT-PCR Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol and the CDC-USA recommendations in a total volume of 10 µL. Negative (extraction control and non-template control [NTC]) and positive controls (RNA extracted from SARS-CoV-2 cell supernatants) were included during all experiments. Primer and probe sequences were synthetized by IDT (Integrated DNA Technologies, Skokie, Illinois, USA). Thermal cycling was performed at 45 °C for 15 min for reverse transcription, followed by 95 °C for 5 min and then 45 cycles of 95 °C for 03 s and 55 °C for 30 s. All experiments were conducted using the Applied Biosystems QuantStudio 5 Real-Time PCR Systems (Applied Biosystems, USA). For data analysis, the QuantStudio software v1.5 was used with baseline and threshold automatic.

Cells

African monkey green kidney-derived cell line Vero CCL-81 was used for virus isolation from positive environmental samples. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM), high glucose (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin

and 100 µg/ml streptomycin (Gibco, USA); and maintained maintained in a humidified atmosphere, at 37 °C and 5% CO_2.

SARS-CoV-2 isolation

Vero CCL-81 cells were cultured in 12-well plates at a density of 2 x 10^5 cells/well. After 24h, the culture media was removed and cells were incubated with 300 µL of undiluted and filtered surface samples at 37°C, 5% CO_2, for 1h. Fresh media supplemented with 2% FBS (700 µL) was added to the cells and they were maintained at 37°C, 5% CO^2. Cells were monitored daily for the visualization of virus-induced cytopathic effect (CPE). CPE images were acquired in Carl Zeiss Axio Observer 5 microscope coupled to a photographic camera. After 3 days post infection (d.p.i.) supernatants were collected and 300 µL were transferred to a new 12-well plate. This procedure was repeated until completing three passages (P1, P2 and P3). Following this, cell culture supernatants were collected on t=0h and t=72h in each passage for viral RNA extraction and possible SARS-CoV-2 detection by RT-qPCR. All experiments were performed in a BSL-3 facility.

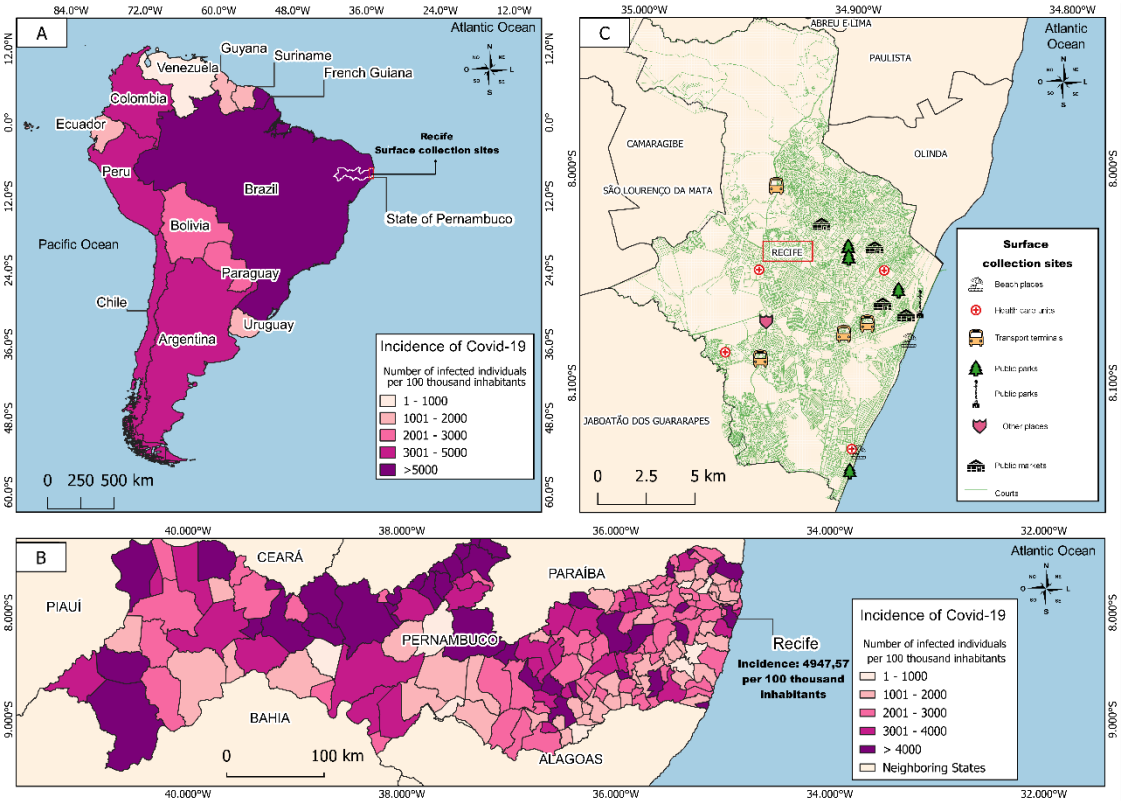
Environmental site assessment questionnaire

Data regarding the social distancing, mask wearing, availability of hand sanitizers and COVID-19 control measures during sample collection in all locations was obtained using a structured questionnaire following the recommendations and guidelines established by WHO and CDC [38]. The questions aimed to identify the implementation and compliance with COVID-19 prevention measures, including social distancing, mask wearing, the availability of hand sanitizers, body temperature measurements for screening and the

285 presence of informative charts for people education. The questionnaires were
286 made with qualitative, with “yes” or “no” input, or quantitative inquiries.

287 **Spatial location of collection surfaces**

288 To georeference the locations where surface samples were obtained, we
289 used the QGIS software (https://qgis.org/en/site/) to generate a map using the
290 geographic coordinates of each publicly available location at
291 https://www.google.com.br/maps. First, we created a graduate map with
292 information about the incidence of COVID-19 in the countries of Latin America
293 (Figure 2A) and all cities located in the State of Pernambuco, Brazil (Figure 2B).
294 The incidence per 100 thousand inhabitants was calculated using the database
295 of the last Brazilian census available at http://censo2010.ibge.gov.br and
296 epidemiological reports of COVID-19 cases from the Pernambuco State Health
297 Department [36] and the World Organization Health (WHO)[39]. Furthermore, we
298 showed the spatial distribution of urban public places where the samples were
299 collected including transport terminals, health care units, public parks, public
300 markets, beach areas, and other areas. We acquired the cartographic base in
301 shapefile format through the Brazilian Institute of Geography and Statistics
302 (IBGE) in the Geocentric Reference System for the Americas (SIRGAS) 2000
303 (Figure 2C).



304

305 **Figure 2. Spatial distribution of surface collection points and incidence of**
306 **COVID-19 in Latin America and Pernambuco state, Brazil.** Fig. 2A shows the
307 incidence of COVID-19 per 100,000 inhabitants in Latin America. Fig. 2B shows the
308 incidence of COVID-19 per 100,000 inhabitants in all cities in the state of Pernambuco,
309 Northeast Brazil. Fig. 1C shows the spatial distribution of surface collection points
310 (transport terminals, health care units, public parks, public markets, beach areas and
311 other places) across Recife, Pernambuco state, Brazil.

312

313 **Data analysis**

314 GraphPad Prism software version 5.01 for Windows (GraphPad
315 Software, La Jolla, California, USA) was used to plot most graphics. The
316 association analysis between collection locations and type of materials was
317 demonstrated based on the results from 97 positive surfaces collected in this

318 study using the web-based Circos table viewer, version 0.63-9
319 (https:www.mkweb.bcgsc.ca/tableviewer/visualize/) [40].

320 **Ethics approval**

321 This study was reviewed and approved under protocol number 03/2021
322 by the Fiocruz Pernambuco Internal Biosafety Commission, as part of quality
323 assurance for working with highly pathogenic virus.

324

325

326

327

328

329

330

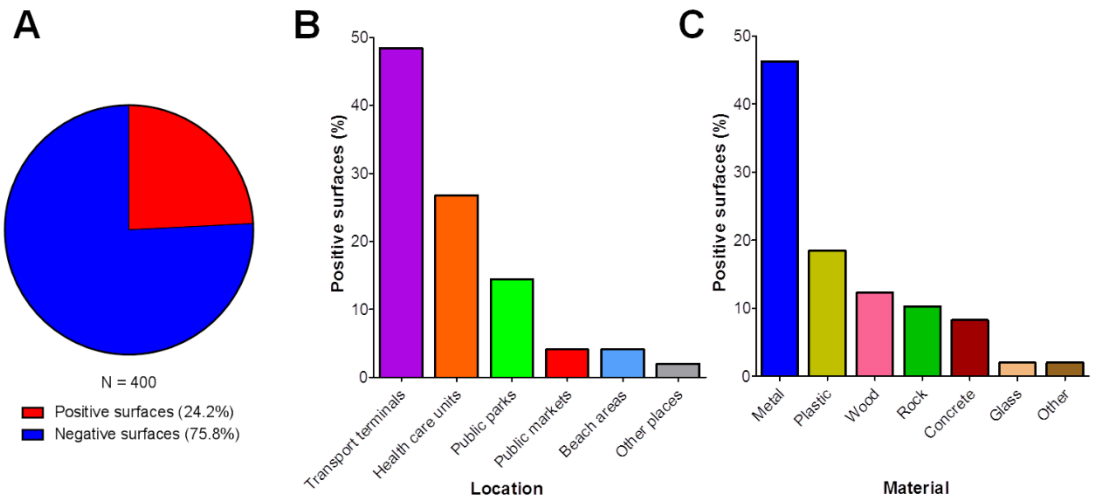
331

332

RESULTS

Distribution of surface samples according to collection area and type of material

A total of 400 surface samples were collected in Recife, Pernambuco state in 19 sites divided into 6 subgroups (health care units, transport terminals, public parks, public markets, beach areas, and a food distribution center). A total of 97 surface samples (24.2%) tested positive for SARS-CoV-2 RNA using the CDC-USA protocol by RT-qPCR (Figure 3a, Supplementary Table 1) in 18 out of 19 sites sampled (Supplementary Table 2). The only site that tested negative was a public market. SARS-CoV-2 RNA was detected in 47 (48.4%) surface samples collected around transport terminals, followed by health care units (26/97, 26.8%), public parks (14/97, 14.4%), public markets (4/97, 4.1%), beach areas (4/97, 4.1%), and other places (2/2.0%) (Figure 3b, Supplementary Table 3). Regarding the type of material where environmental samples were collected, SARS-CoV-2 RNA was found most frequently on metal (45/97, 46.3%), followed by plastic (18/97, 18.5%), wood (12/97, 12.3%), rock (10/97, 10.3%), concrete (8/97, 8.2%), glass (2/97, 2.0%), and other (ceramic and rubber) (2/97, 2.0%) (Figure 3c). Positive samples were predominantly found in toilets, ATMs, handrails, playground, and outdoor gym; highlighting the importance of these fomites in SARS-CoV-2 surface contamination.



353

354 **Figure 3. Overall results for SARS-CoV-2 detection in surface samples.** Fig. 3A
355 shows the distribution of positive and negative samples using a total of 400
356 environmental samples. Fig. 3B shows the distribution of positive samples according to
357 the collection areas; including transport terminals, health care units, public parks, public
358 markets, beach areas, and other places Fig. 3C shows the distribution of positive
359 samples according to the type of material including metal, plastic, wood, rock, concrete,
360 glass and other.

361

362 **Distribution of positive surface samples according to point of collection**

363 ***Transport terminals***

364 Forty-seven (48.4%) surface samples were positive for SARS-CoV-2
365 RNA around public transport terminals with Cq values ranging from 31.1 to 38.7
366 by RT-qPCR (Supplementary Table 3). Positive samples were distributed
367 particularly in eleven different locations, including ATM (9/47, 19.1%), handrails
368 (9/47, 19.1%), bus terminal access (7/47, 14.8%), bench (6/47, 12.7%), toilet
369 (5/47, 10.6%), ticket machine (3/47, 6.3%), bus stop (2/47, 4.2%), subway

station access (2/47, 4.2%), faucet (2/47, 4.2%), bus terminal exit (1/47, 2.1%), and ticket counter (1/47, 2.1%) (Figure 4a, Supplementary Table 3).

Health care units

Twenty-six (26.8%) surface samples were positive for SARS-CoV-2 RNA in the surroundings of health care units with Cq values ranging from 31.1 to 38.7 by RT-qPCR (Supplementary Table 3). Positive samples were found in nine different locations from four reference hospitals for COVID-19 treatment. The areas with highest number of positive samples were hospital access (10/26, 38.4%), bus stop (4/26, 15.3%), traffic light button (4/26, 15.3%), principal entrance (2/26, 7.6%), resting area (2/26, 7.6%), toilet (1/26, 3.8%), ambulatory entrance (1/26, 3.8%), coffee shop (1/26, 3.8%), and public phone (1/26, 3.8%) (Figure 4b, Supplementary Table 3).

Public parks

Fourteen (14.4%) surface samples were positive for SARS-CoV-2 RNA around public parks, with Cq values ranging from 36.2 to 39.7 by RT-qPCR (Supplementary Table 3). Positive samples were collected from five different locations, including playground (5/14, 35.7%), recreation area (4/14, 28.5%), outdoor gym (2/14, 14.2%), toilet (2/14, 14.2%), and handrails (1/14, 7.1%) (Figure 4c, Supplementary Table 3). There were no positive samples from the public bike station, bus stop, coffee shop, traffic light button, or faucet.

Public markets

Three out of four public markets sampled returned at least one positive sample. Four (4.1%) surface samples were positive for SARS-CoV-2 RNA in

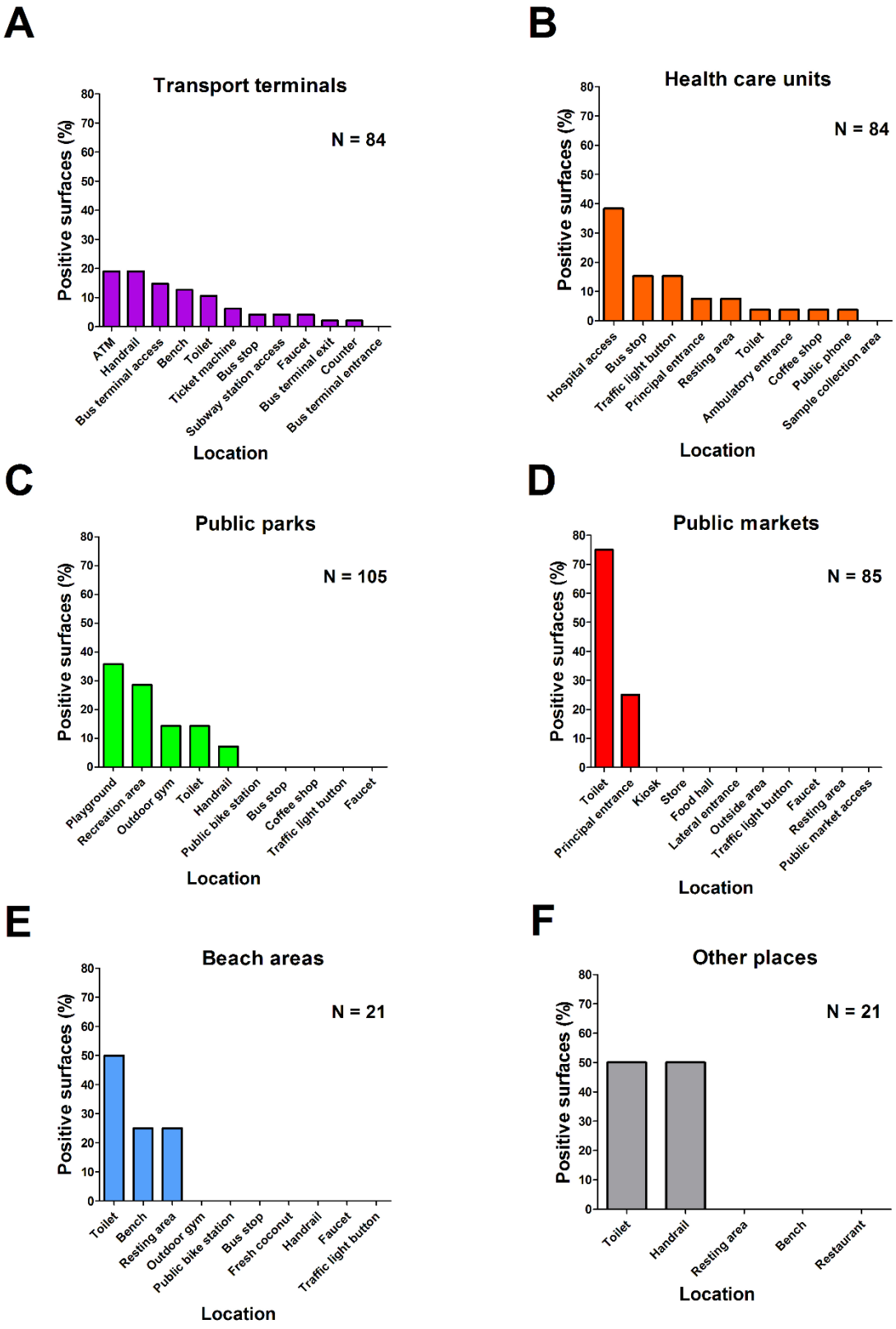
public markets with Cq values ranging from 36.9 to 38.1 by RT-qPCR (Supplementary Table 3). Positive samples were collected from two different locations, including toilets (3/4, 75.0%) and principal entrance (1/4, 25.0%) (Figure 4d, Supplementary Table 3). No positive samples were found at the kiosk, store, lateral entrance, outside area, food hall, public market access, traffic light button, faucet, or resting area.

Beach areas

Four (4.1%) surface samples were positive for SARS-CoV-2 RNA in beach areas with Cq values ranging from 36.1 to 37.9 by RT-qPCR (Supplementary Table 3). Positive samples were collected from three different locations, including toilets (2/4, 50.0%), bench (1/4, 25.0%), and resting area (1/4, 25.0%) (Figure 4e, Supplementary Table 3). No positive samples were detected from the outdoor gym, public bike station, bus stop, fresh coconut, handrail, faucet, or traffic light button.

Other places

Two (2.0%) surface samples were positive for SARS-CoV-2 RNA around one food distribution center with Cq values ranging from 38.0 to 38.7 by RT-qPCR (Supplementary Table 3). Positive samples were collected from two different locations, including toilet (1/2, 50.0%) and handrails (1/2, 50.0%) (Figure 4f, Supplementary Table 3). No positive samples were found in restaurants or resting benches.



414

415 **Figure 4. Distribution of positive surface samples according to collection areas.**

416 Fig. 4A shows the distribution of positive samples around transport terminals. Fig. 4B

shows the distribution of positive samples around health care units. Fig. 4C shows the distribution of positive samples around public parks. Fig. 4D shows the distribution of positive samples around public markets. Fig. 4E shows the distribution of positive samples around beach areas. Fig. 4F shows the distribution of positive samples around the other areas (including one food distribution center).

Types of surface materials positive for SARS-CoV-2 RNA

From the 47 positive samples in transport terminals, 21 (44.6%) samples were identified mainly on metal surfaces, especially from handrails at bus terminals, ATM button, protection grid, and faucet. 19 (19.1%) samples were recovered from plastic surfaces, especially around biometrics sensors in ATMs and faucets in the toilet. 5 (10.6%) samples were found in concrete surfaces, most being found in pillars near the bus stop and one sampled from a bench. Four (8.5%) samples were collected on rock surfaces, with virus being detected on walls in the toilet and bus terminal, and one sample was collected at the terminal service desk. Four (8.1%) samples were identified on wood surfaces, all being from benches near the bus stop of transport terminals. Two (4.2%) samples were detected on glass surfaces, mainly on the ticket machine screens. In addition, one (2.1%) sample was collected on a toilet seat (porcelain) and one (2.1%) was detected on the ticket machine (rubber) (Figure 5, Supplementary Table 3).

From the 26 positive samples found in health care units and neighboring areas, 12 (46.1%) samples were recovered from metal surfaces mostly, located at the entrance to hospitals and near bus stops. 7 (26.9%) samples were

441 identified in plastic surfaces, especially from traffic light buttons, near bus stops,
442 and in the toilets. Four (15.3%) samples were detected in rock surfaces found at
443 the entrance to hospitals. Two (7.6%) samples were identified in wood surfaces
444 at the entrance to hospitals. One (3.8%) sample was detected on the concrete
445 surface from a nearby bus stop (Figure 5, Supplementary Table 3).

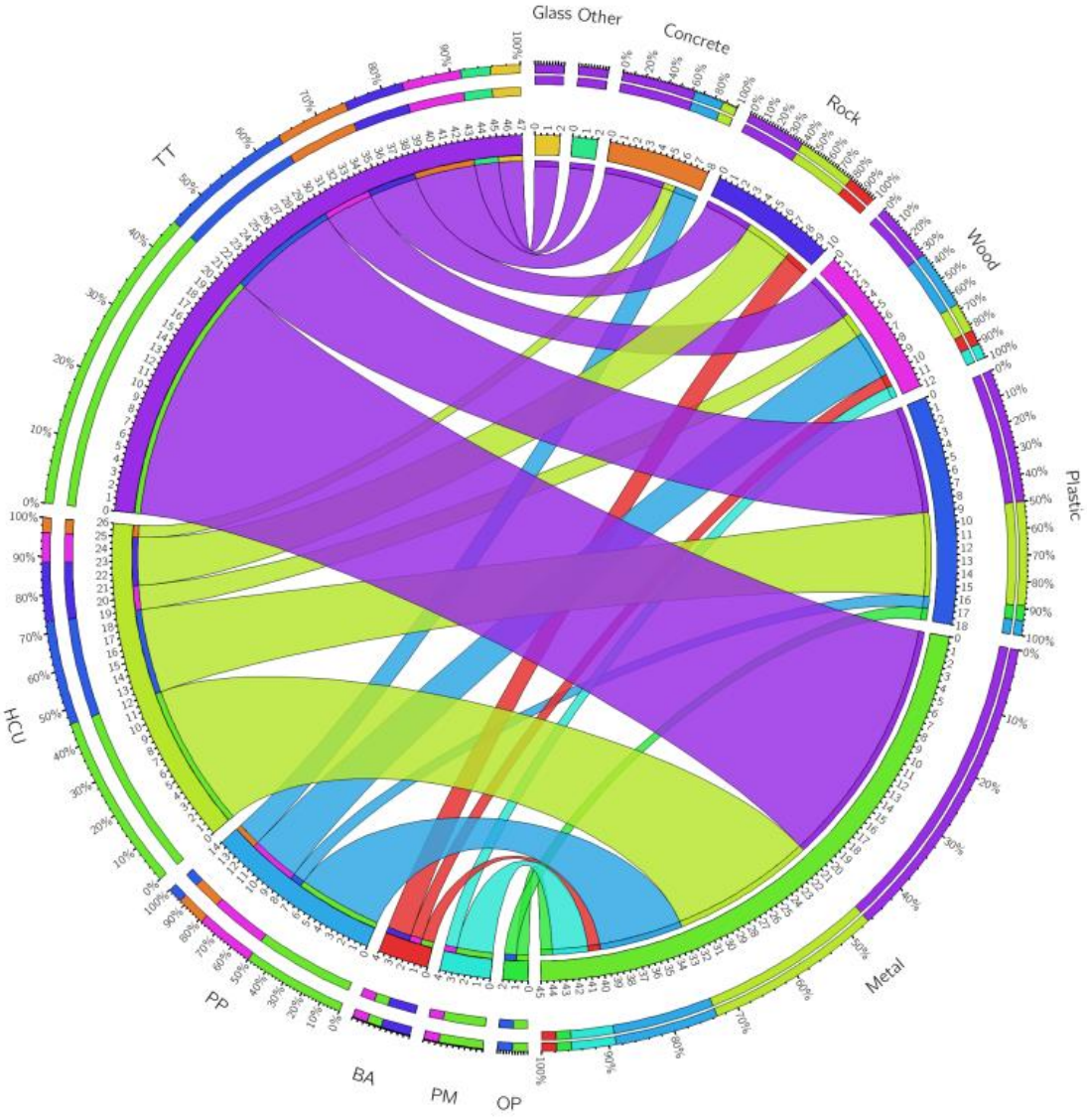
446 From the 14 positive samples found in public parks, seven (50.0%)
447 samples were identified on the metal surfaces of handrails in the playground
448 and outdoor gym. Four (28.5%) samples were recovered from wood surfaces in
449 the playground, and one tourist attraction point. Two (14.2%) samples were
450 detected in concrete surfaces of the playground. One (7.1%) sample was
451 identified in plastic surface from a faucet in the toilet (Figure 5, Supplementary
452 Table 3).

453 From the four positive samples in public markets, three (75.0%) samples
454 were detected on metal surfaces at the entrance to public markets, and from a
455 toilet faucet. One (25.0%) sample was detected identified in wood surfaces from
456 a door in the toilet.

457 From the four positive samples in beach areas, two (50.0%) were
458 detected in rock surfaces, one from toilet wall and one from a bench. One
459 (25.0%) sample was identified in a metal surface from a faucet in the toilet, and
460 a further one (25.0%) was detected in a wood surface on a handrail that gives
461 access to the beach.

462 Lastly, of the two positive samples from two food distribution center, one
463 (50.0%) sample was detected on a plastic surface from a faucet in the toilet and

464 one (50.0%) was identified on a metal handrail surface at the entrance of a
465 bank (Figure 5, Supplementary Table 3).



466

467 **Figure 5. Association between the surface collection areas, and type of material**
468 **where SARS-CoV-2 RNA was detected.** TT: transport terminals; HCU: health care
469 units; PP: public parks; PM: public markets; BA: beach areas; OP: other places.

470

471

472 **Viability of SARS-CoV-2 from positive surfaces samples**

To assess infectivity of samples that tested positive by RT-qPCR, nine samples with Cq value <34 (Cq ranging from 31.0 to 33.7) were inoculated into 12-well plates seeded with Vero CCL-81 cells. Samples were considered negative after three blind passages of the supernatant. Under these conditions it was not possible to isolate the virus, as determined by the absence of CPE and negative RT-qPCR results from third passage supernatant (Supplementary Figure 2, Supplementary Table 4). The risk of infection from these contaminated surfaces is therefore not clear.

Poor adherence of COVID-19 mitigation measures by society

Data regarding the adoption of public health measures, and community perception of COVID-19 disease was collected during surface collection in all locations by using a structured environmental site assessment questionnaire. In the 19 collection points, 70% alcohol-based hand sanitizer was available at the entrance in 26.3% (5/19) of the locations, whereas 42.1% (8/19) had a sink with soap and water for hand hygiene. Temperature measurements at the entrance was carried out in 15.8% (3/19) of the sites, and information material on preventive measures to prevent SARS-CoV-2 transmission was found in 42.1% (8/19) of the sites. High mask wear adherence was seen (94.7% [18/19]), although only 57.3% of people (average calculated for every 10 people per collection point) were wearing masks in a proper way. Regarding social distancing, only 26.3% (5/19) of the people present at collection points were maintaining the recommended social distance of 2 m. Furthermore, only 5.3% (1/19) of collection sites were limiting the number of people who accessed the location point (Table 1). We found no positive correlation between adherence of COVID-19 mitigation measures and SARS-CoV-2 positivity (data not shown).

498 Overall, our findings indicated poor adherence of COVID-19 mitigation
499 measures in our study areas.

500

501

502

503

504

505

506

507

508

509

510

511

512

513

514

515

516

DISCUSSION

Since the emergence of SARS-CoV-2, first identified in China, the highly pathogenic coronavirus has spread rapidly around the world causing an unprecedented health security crisis and drastically affecting the global economic stability. Thus, understanding the modes of transmission of SARS-CoV-2 among humans is a critical step to establish effective prevention policies and prioritize resources to break the chain of SAR-CoV-2 transmission. The transmission through direct contact and via airborne (respiratory droplets and/or aerosols) are pointed as the dominant routes for the transmission of SARS-CoV-2 in humans [21, 22, 41] and animal models, like ferrets [42], golden hamsters [43], and mices[44]. Similarly, many studies conducted on the spread of other respiratory viruses, including influenza virus [45, 46], respiratory syncytial virus (RSV) [47], and severe acute respiratory syndrome coronavirus (SARS-CoV-1) [48], evidenced that these respiratory viruses can be exhaled and transmitted via airborne. However, the transmission dynamics of SARS-CoV-2 by environmental surfaces and their role in the transmission chain remains unclear and may be multifactorial, especially in urban areas with a large flow and concentrations of people with real-life challenges. Here, we investigated the presence of SARS-CoV-2 RNA on public high-touch surfaces in a large metropolitan city during the second wave of the COVID-19 pandemic in Brazil.

A recent study investigated the presence of SARS-CoV-2 RNA on public surfaces in Belo Horizonte, a large city with a tropical savanna climate in Southeast Brazil. A total of 933 swabs collected from different locations including health care units, public squares, bus terminals, public markets, and other public places between April and June 2020[49]. The results showed that 49

(5.25%) of surface samples were tested positive for SARS-CoV-2 RNA, although the infectious potential of positive samples was not investigated. Considering the proportion of positivity in the different places, the authors pointed out that bus terminals exhibited the highest positivity rate, followed by public markets, public squares, and health care units [49]. In our study, we found higher positivity of SARS-CoV-2 RNA (97/400, 24.2%) detection of surfaces compared to the Belo Horizonte survey. In our study, most of the positive samples in our study were detected in the surroundings of transport terminals areas (48.4%), followed by health care units (26.8%), public parks (14.4%), public markets (4.1%), and beach areas (4.1%). The difference in the positivity rate of both cities cannot be explained by climate differences as Recife is hotter and more humid than Belo Horizonte, conditions that decreases the stability of SARS-CoV-2 in the environment [50] and its transmissibility[51]. A more plausible explanation for this disparity is the number of confirmed COVID-19 cases in these cities by the time of sample collection. Whereas Belo Horizonte registered 400 to 5,000 (https://ciis.fmrp.usp.br/covid19/bh-mg/) daily cases between April and June 2020, Recife had 60,000 to 70,000 (https://ciis.fmrp.usp.br/covid19/recife-pe/) in February 2021. Taken together, our findings are in agreement with others and indicates widespread SARS-CoV-2 surface contamination in public urban places with a large flow of people [49, 52].

Regarding the distribution of positive samples according to the type of material, we found the SARS-CoV-2 RNA mainly on metal, followed by plastic, wood, rock, concrete, and glass. Similarly, a recent urban study found the SARS-CoV-2 RNA on different types of materials, the majority on metal, concrete, rock, brickwork, wood, and glass[49]. Interestingly, our data

567 demonstrated that the positive samples for SARS-CoV-2 RNA were mainly
568 collected in toilets. These findings also corroborate data obtained by other
569 groups[26, 30, 53], which toilets as an area of high positivity rate for SARS-CoV-2
570 RNA. Additionally, our findings revealed other specific locations with high rates
571 of positivity: ATMs, handrails, playgrounds, and outdoor gyms.

572 Previous studies performed under controlled laboratory conditions have
573 shown that SARS-CoV-2 remains infectious on different types of surfaces, such
574 as stainless steel, glass and paper, for up to 28 days at 20 °C[34], depending on
575 type of environmental surface; and can remain viable in aerosols for up to 3 h
576 [35]. Notably, the viral load decreases over time and depends on the length of
577 time since the virus has been deposited on the surface, which may be reflected
578 in the presence of infectious or non-infectious viral particles and, consequently,
579 infection risk in humans [21, 23, 24]. Another important factor that must be
580 considered is the minimal infectious dose of SARS-CoV-2 to start an effective
581 infection in humans, which has not yet been clarified. In order to elucidate the
582 transmission dynamics of SARS-CoV-2 by environmental surfaces in real-life
583 conditions, several studies have investigated the presence of SARS-CoV-2 in
584 air and environmental surfaces/areas, including health care settings [25-31, 33] and
585 urban settings[49, 52-55]. In general, these studies have found varying levels of
586 environmental contamination, ranging from extensive [25, 26] to low contamination
587 [31, 49], or even no contamination of SARS-CoV-2 RNA. However, many of these
588 studies did not determine the ability of SARS-CoV-2 to be cultured from such
589 environmental swabs, which would help to understand the implications of
590 SARS-CoV-2 RNA positive environmental samples in terms of infectious
591 potential for the human population [25, 27]. In this study, we evaluated the

592 infectious potential of positive surface samples (Cq value <34) in Vero CCL-81
593 cells, but SARS-CoV-2 could not be cultured. This finding is supported by
594 recent studies, which have demonstrated the low potential infectious from the
595 environmental swabs using cell culture [25, 31, 56]. This may explain the lack of
596 success in virus isolation given the short half-life of SARS-CoV-2 in the
597 environment. Serial sampling of highly touched surfaces in places with large
598 people flow might produce culturable SARS-CoV-2. Nevertheless, our findings
599 identify the locations and objects that pose the highest risk of contamination
600 through fomites and should be considered as COVID-19 critical control points.
601 The difficulty in culturing viruses from environmental samples arises from low
602 viral load concentrations and instability of SARS-CoV-2 outside the human host.
603 Recent studies aggregated environmental sampling has shown high RT-qPCR
604 Cq values (>30) for most of the positive samples, which may explain the
605 difficulty of SARS-CoV-2 to be cultured from the environmental specimens [25, 33,
606 49]. Other studies have suggested that several environmental stressors can
607 compromise and damage the integrity of SARS-CoV-2 viral particles, including
608 temperature and relative humidity [34, 50].

609 SARS-CoV-2 contamination of public surfaces suggests the circulation of
610 infected people and the risk of infection in these locations either by direct or
611 indirect contact with infected patients. Direct contact with an infectious source is
612 important for the establishment of COVID-19 clinical features and this has been
613 established using animal models. Transmission studies in the ferret SARS-CoV-
614 2 model have demonstrated that airborne transmission is likely but is
615 considerably less efficient than direct contact transmission, whereby direct

616 contacting animals are exposed to infected ferrets and share with them the
617 same food, water, bedding, and breathe the same air[42, 57].

618 Regarding the adherence of COVID-19 mitigation measures by society, a
619 number of studies have been performed in order to evaluate the adoption of
620 measures to prevent the SARS-CoV-2 transmission [58-60]. To assess the
621 community's adherence to mitigation measures to combat the rapid spread of
622 SARS-CoV-2, a recent cross-sectional study conducted in Malaysia employed
623 4,850 Malaysian residents, between 27th March and 3rd April 2020 [59]. The
624 findings revealed that most participants (83.1%) held positive attitudes toward
625 the successful control of COVID-19, the capacity of Malaysia to counter rapid
626 spread of the disease (95.9%) and the way the Malaysian government was
627 facing the COVID-19 crisis (89.9%). Furthermore, most participants were also
628 taking precautions such as practicing hand hygiene (87.8%) and avoiding large
629 gatherings (83.4%) [59]. Interestingly, the number of COVID-19 cases in Malaysia
630 remained stable, with a progressive increase observed only between
631 September and November 2020 (https://ourworldindata.org/covid-cases). In
632 contrast, a community-based cross-sectional study done in Northeast Ethiopia,
633 evaluated the adherence towards COVID-19 mitigation strategies by society
634 among 635 individuals from April 20–27, 2020 [58]. The results showed that
635 approximately half of the study participants had poor adherence towards
636 COVID-19 mitigation measures. In the current analysis, although the number of
637 places evaluated was limited (19), it is important to highlight that these are
638 places with a high flow and concentration of people. Our data demonstrated low
639 adherence of COVID-19 mitigation measures by society regarding the social
640 distancing, effective use of masks, precaution measures adoption and

641 community's perception about the COVID-19 disease. Taken together, these
642 results highlight the importance of consistent messaging from government and
643 health authorities to improve levels the adoption of measures to prevent and
644 contain the spread of SARS-CoV-2.

645 In summary, our data demonstrated the extensive viral RNA
646 contamination of surfaces in a range of public urban settings in the absence of
647 viral isolation, which suggests low potential risk from environmental
648 contamination for the human population. However, we identified poor
649 adherence to COVID-19 mitigation policies by wider society regarding the
650 adoption of control measures, and this may be reflected in the frequent
651 detection of the viral RNA. Studies such as these can contribute to assess the
652 prevalence of SARS-CoV-2 in specific settings. Finally, we suggest that further
653 studies are urgently performed to elucidate the relative contribution of various
654 modes of transmission for SARS-CoV-2 in both healthcare and urban-settings.

655 **Funding**

656 The work in Dr. Pena's lab is funded by the Fiocruz Inova Program, IDRC-
657 Canada and the Foundation for Science and Technology of Pernambuco –
658 FACEPE, Brazil (grant number APQ-0560-2.12/19). S.J.R.d.S. is supported by
659 a PhD fellowship sponsored by the Foundation for Science and Technology of
660 Pernambuco (FACEPE), reference number IBPG-1321-2.12/18. A.K. is funded
661 by the UK Medical Research Council (MC_UU_12014/8). The funders had no
662 role in study design, data collection and analysis, decision to publish, or
663 preparation of the manuscript.

664 **Acknowledgments**

We are grateful to Msc. Larissa Krokovsky for her support and critical analysis for the design of the work. We thank Josenildo do Sobral for helping to collect surface samples. We would like to express our sincere gratitude and appreciation to the Fiocruz Pernambuco team for the support.

Authorship contribution statement

L.P., A.K. and S.J.R.d.S. conceived the work. S.J.R.d.S., J.C.F.N., W.P.M.d.S.R., C.T.A.S., P.G.S., R.P.G.M., A.A.M., B.N.R.S. and J.J.F.M. performed the experiments. S.J.R.d.S., J.C.F.N., W.P.M.d.S.R., P.G.S. A.A.M., J.J.F.M., A.K. and L.P. performed data analysis and interpretation. S.J.R.d.S., J.C.F.N., W.P.M.d.S.R., A.A.M. and J.J.F.M. wrote the original draft. S.J.R.d.S., A.K. and L.P. wrote the final manuscript. LP supervised the work. All authors critically revised the manuscript and approved the final version of the submitted manuscript.

Competing interests

The authors declare no competing interests.

681 **Table 1.** Evaluation of safety procedure protocol implementation against COVID-19 at
682 collection areas (n=19).

Variables	Frequency	Percent (%)
Availability of 70% alcohol at the entrance		
Yes	5	26.3
No	14	73.7
Availability of faucets and soap for handwashing		
Yes	8	42.1
No	11	57.9
Temperature measurement at the entrance location		
Yes	3	15.8
No	16	84.2
Availability of informative material on preventive measures against COVID-19		
Yes	8	42.1
No	11	57.9
People wearing mask		
Yes	18	94.7
No	1	5.3
Social distancing[a]		
Yes	5	26.3
No	14	73.7
Control of the number of persons accessing the area		
Yes	1	5.3
No	18	94.7

683 [a] considering 2 m

References

1. Fehr, A. R.; Perlman, S., Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* **2015,** *1282*, 1-23.
2. Cui, J.; Li, F.; Shi, Z. L., Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* **2019,** *17* (3), 181-192.
3. Zhong, N. S.; Zheng, B. J.; Li, Y. M.; Poon; Xie, Z. H.; Chan, K. H.; Li, P. H.; Tan, S. Y.; Chang, Q.; Xie, J. P.; Liu, X. Q.; Xu, J.; Li, D. X.; Yuen, K. Y.; Peiris; Guan, Y., Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* **2003,** *362* (9393), 1353-8.
4. Zaki, A. M.; van Boheemen, S.; Bestebroer, T. M.; Osterhaus, A. D.; Fouchier, R. A., Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* **2012,** *367* (19), 1814-20.
5. Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; Bi, Y.; Ma, X.; Zhan, F.; Wang, L.; Hu, T.; Zhou, H.; Hu, Z.; Zhou, W.; Zhao, L.; Chen, J.; Meng, Y.; Wang, J.; Lin, Y.; Yuan, J.; Xie, Z.; Ma, J.; Liu, W. J.; Wang, D.; Xu, W.; Holmes, E. C.; Gao, G. F.; Wu, G.; Chen, W.; Shi, W.; Tan, W., Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* **2020,** *395* (10224), 565-574.
6. Zhou, P.; Yang, X. L.; Wang, X. G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H. R.; Zhu, Y.; Li, B.; Huang, C. L.; Chen, H. D.; Chen, J.; Luo, Y.; Guo, H.; Jiang, R. D.; Liu, M. Q.; Chen, Y.; Shen, X. R.; Wang, X.; Zheng, X. S.; Zhao, K.; Chen, Q. J.; Deng, F.; Liu, L. L.; Yan, B.; Zhan, F. X.; Wang, Y. Y.; Xiao, G. F.; Shi, Z. L., A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **2020,** *579* (7798), 270-273.
7. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; Niu, P.; Zhan, F.; Ma, X.; Wang, D.; Xu, W.; Wu, G.; Gao, G. F.; Tan, W.; Team, C. N. C. I. a. R., A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **2020,** *382* (8), 727-733.
8. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; Cheng, Z.; Yu, T.; Xia, J.; Wei, Y.; Wu, W.; Xie, X.; Yin, W.; Li, H.; Liu, M.; Xiao, Y.; Gao, H.; Guo, L.; Xie, J.; Wang, G.; Jiang, R.; Gao, Z.; Jin, Q.; Wang, J.; Cao, B., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **2020,** *395* (10223), 497-506.
9. Dong, E.; Du, H.; Gardner, L., An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* **2020,** *20* (5), 533-534.
10. Faria, N. R.; Mellan, T. A.; Whittaker, C.; Claro, I. M.; Candido, D. D. S.; Mishra, S.; Crispim, M. A. E.; Sales, F. C. S.; Hawryluk, I.; McCrone, J. T.; Hulswit, R. J. G.; Franco, L. A. M.; Ramundo, M. S.; de Jesus, J. G.; Andrade, P. S.; Coletti, T. M.; Ferreira, G. M.; Silva, C. A. M.; Manuli, E. R.; Pereira, R. H. M.; Peixoto, P. S.; Kraemer, M. U. G.; Gaburo, N.; Camilo, C. D. C.; Hoeltgebaum, H.; Souza, W. M.; Rocha, E. C.; de Souza, L. M.; de Pinho, M. C.; Araujo, L. J. T.; Malta, F. S. V.; de Lima, A. B.; Silva, J. D. P.; Zauli, D. A. G.; Ferreira, A. C. S.; Schnekenberg, R. P.; Laydon, D. J.; Walker, P. G. T.; Schlüter, H. M.; Dos Santos, A. L. P.; Vidal, M. S.; Del Caro, V. S.; Filho, R. M. F.; Dos Santos, H. M.; Aguiar, R. S.; Proença-Modena, J. L.; Nelson, B.; Hay, J. A.; Monod, M.; Miscouridou, X.; Coupland, H.; Sonabend, R.; Vollmer, M.; Gandy, A.; Prete, C. A.; Nascimento, V. H.; Suchard, M. A.; Bowden, T. A.; Pond, S. L. K.; Wu, C. H.; Ratmann, O.; Ferguson, N. M.; Dye, C.; Loman, N. J.; Lemey, P.; Rambaut, A.; Fraiji, N. A.; Carvalho, M. D. P. S.; Pybus, O. G.; Flaxman, S.; Bhatt, S.; Sabino, E. C., Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* **2021**.
11. Abdool Karim, S. S.; de Oliveira, T., New SARS-CoV-2 Variants - Clinical, Public Health, and Vaccine Implications. *N Engl J Med* **2021**.

12. Naveca, F. G.; Nascimento, V.; de Souza, V. C.; Corado, A. L.; Nascimento, F.; Silva, G.; Costa, Á.; Duarte, D.; Pessoa, K.; Mejía, M.; Brandão, M. J.; Jesus, M.; Gonçalves, L.; da Costa, C. F.; Sampaio, V.; Barros, D.; Silva, M.; Mattos, T.; Pontes, G.; Abdalla, L.; Santos, J. H.; Arantes, I.; Dezordi, F. Z.; Siqueira, M. M.; Wallau, G. L.; Resende, P. C.; Delatorre, E.; Gräf, T.; Bello, G., COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med* **2021**.
13. Peacock, T. P.; Penrice-Randal, R.; Hiscox, J. A.; Barclay, W. S., SARS-CoV-2 one year on: evidence for ongoing viral adaptation. *J Gen Virol* **2021,** *102* (4).
14. Marquès, M.; Domingo, J. L., Contamination of inert surfaces by SARS-CoV-2: Persistence, stability and infectivity. A review. *Environ Res* **2021,** *193*, 110559.
15. Hu, B.; Guo, H.; Zhou, P.; Shi, Z. L., Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* **2021,** *19* (3), 141-154.
16. Kutter, J. S.; Spronken, M. I.; Fraaij, P. L.; Fouchier, R. A.; Herfst, S., Transmission routes of respiratory viruses among humans. *Curr Opin Virol* **2018,** *28*, 142-151.
17. Tang, J. W.; Nicolle, A. D.; Klettner, C. A.; Pantelic, J.; Wang, L.; Suhaimi, A. B.; Tan, A. Y.; Ong, G. W.; Su, R.; Sekhar, C.; Cheong, D. D.; Tham, K. W., Airflow dynamics of human jets: sneezing and breathing - potential sources of infectious aerosols. *PLoS One* **2013,** *8* (4), e59970.
18. Leung, N. H. L.; Chu, D. K. W.; Shiu, E. Y. C.; Chan, K. H.; McDevitt, J. J.; Hau, B. J. P.; Yen, H. L.; Li, Y.; Ip, D. K. M.; Peiris, J. S. M.; Seto, W. H.; Leung, G. M.; Milton, D. K.; Cowling, B. J., Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat Med* **2020,** *26* (5), 676-680.
19. Stadnytskyi, V.; Bax, C. E.; Bax, A.; Anfinrud, P., The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. *Proc Natl Acad Sci U S A* **2020,** *117* (22), 11875-11877.
20. Kaur, S.; Bherwani, H.; Gulia, S.; Vijay, R.; Kumar, R., Understanding COVID-19 transmission, health impacts and mitigation: timely social distancing is the key. *Environ Dev Sustain* **2020**, 1-17.
21. Zhang, R.; Li, Y.; Zhang, A. L.; Wang, Y.; Molina, M. J., Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proc Natl Acad Sci U S A* **2020,** *117* (26), 14857-14863.
22. Falahi, S.; Kenarkoohi, A., Transmission routes for SARS-CoV-2 infection: review of evidence. *New Microbes New Infect* **2020,** *38*, 100778.
23. Chin, A. W. H.; Chu, J. T. S.; Perera, M. R. A.; Hui, K. P. Y.; Yen, H. L.; Chan, M. C. W.; Peiris, M.; Poon, L. L. M., Stability of SARS-CoV-2 in different environmental conditions. *Lancet Microbe* **2020,** *1* (1), e10.
24. van Doremalen, N.; Bushmaker, T.; Morris, D. H.; Holbrook, M. G.; Gamble, A.; Williamson, B. N.; Tamin, A.; Harcourt, J. L.; Thornburg, N. J.; Gerber, S. I.; Lloyd-Smith, J. O.; de Wit, E.; Munster, V. J., Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N Engl J Med* **2020**.
25. Zhou, J.; Otter, J. A.; Price, J. R.; Cimpeanu, C.; Garcia, D. M.; Kinross, J.; Boshier, P. R.; Mason, S.; Bolt, F.; Holmes, A. H.; Barclay, W. S., Investigating SARS-CoV-2 surface and air contamination in an acute healthcare setting during the peak of the COVID-19 pandemic in London. *Clin Infect Dis* **2020**.
26. Chia, P. Y.; Coleman, K. K.; Tan, Y. K.; Ong, S. W. X.; Gum, M.; Lau, S. K.; Lim, X. F.; Lim, A. S.; Sutjipto, S.; Lee, P. H.; Son, T. T.; Young, B. E.; Milton, D. K.; Gray, G. C.; Schuster, S.; Barkham, T.; De, P. P.; Vasoo, S.; Chan, M.; Ang, B. S. P.; Tan, B. H.; Leo, Y. S.; Ng, O. T.; Wong, M. S. Y.; Marimuthu, K.; Team, S. N. C. O. R., Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. *Nat Commun* **2020,** *11* (1), 2800.
27. Santarpia, J. L.; Rivera, D. N.; Herrera, V. L.; Morwitzer, M. J.; Creager, H. M.; Santarpia, G. W.; Crown, K. K.; Brett-Major, D. M.; Schnaubelt, E. R.; Broadhurst, M. J.;

784 Lawler, J. V.; Reid, S. P.; Lowe, J. J., Aerosol and surface contamination of SARS-CoV-2
785 observed in quarantine and isolation care. *Sci Rep* **2020,** *10* (1), 12732.
786 28. Mouchtouri, V. A.; Koureas, M.; Kyritsi, M.; Vontas, A.; Kourentis, L.; Sapounas, S.;
787 Rigakos, G.; Petinaki, E.; Tsiodras, S.; Hadjichristodoulou, C., Environmental contamination of
788 SARS-CoV-2 on surfaces, air-conditioner and ventilation systems. *Int J Hyg Environ Health*
789 **2020,** *230*, 113599.
790 29. Tan, L.; Ma, B.; Lai, X.; Han, L.; Cao, P.; Zhang, J.; Fu, J.; Zhou, Q.; Wei, S.; Wang, Z.;
791 Peng, W.; Yang, L.; Zhang, X., Air and surface contamination by SARS-CoV-2 virus in a tertiary
792 hospital in Wuhan, China. *Int J Infect Dis* **2020,** *99*, 3-7.
793 30. Liu, Y.; Ning, Z.; Chen, Y.; Guo, M.; Gali, N. K.; Sun, L.; Duan, Y.; Cai, J.; Westerdahl,
794 D.; Liu, X.; Xu, K.; Ho, K. F.; Kan, H.; Fu, Q.; Lan, K., Aerodynamic analysis of SARS-CoV-2 in
795 two Wuhan hospitals. *Nature* **2020,** *582* (7813), 557-560.
796 31. Colaneri, M.; Seminari, E.; Novati, S.; Asperges, E.; Biscarini, S.; Piralla, A.; Percivalle,
797 E.; Cassaniti, I.; Baldanti, F.; Bruno, R.; Mondelli, M. U.; Force, C. I. S. M. P. T., Severe acute
798 respiratory syndrome coronavirus 2 RNA contamination of inanimate surfaces and virus
799 viability in a health care emergency unit. *Clin Microbiol Infect* **2020,** *26* (8), 1094.e1-1094.e5.
800 32. Ye, G.; Lin, H.; Chen, S.; Wang, S.; Zeng, Z.; Wang, W.; Zhang, S.; Rebmann, T.; Li,
801 Y.; Pan, Z.; Yang, Z.; Wang, Y.; Wang, F.; Qian, Z.; Wang, X., Environmental contamination of
802 SARS-CoV-2 in healthcare premises. *J Infect* **2020,** *81* (2), e1-e5.
803 33. Dargahi, A.; Jeddi, F.; Vosoughi, M.; Karami, C.; Hadisi, A.; Ahamad Mokhtari, S.;
804 Ghobadi, H.; Alighadri, M.; Haghighi, S. B.; Sadeghi, H., Investigation of SARS CoV-2 virus in
805 environmental surface. *Environ Res* **2021,** *195*, 110765.
806 34. Riddell, S.; Goldie, S.; Hill, A.; Eagles, D.; Drew, T. W., The effect of temperature on
807 persistence of SARS-CoV-2 on common surfaces. *Virol J* **2020,** *17* (1), 145.
808 35. van Doremalen, N.; Bushmaker, T.; Morris, D. H.; Holbrook, M. G.; Gamble, A.;
809 Williamson, B. N.; Tamin, A.; Harcourt, J. L.; Thornburg, N. J.; Gerber, S. I.; Lloyd-Smith, J. O.;
810 de Wit, E.; Munster, V. J., Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-
811 CoV-1. *N Engl J Med* **2020,** *382* (16), 1564-1567.
812 36. SAÚDE. BOLETIM COVID-19 - COMUNICAÇÃO SES-PE 2021.
813 https://drive.google.com/file/d/1F4EpduaUOx622KQAZARg6Saq1WU0L2Xj/vie
814 w.
815 37. CDC. Research use only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR
816 primers and probes *WHO* [Online], 2020. https://www.cdc.gov/coronavirus/2019-
817 ncov/lab/rt-pcr-panel-primer-probes.html.
818 38. CDC. How to protect yourself & others 2021.
819 https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-
820 sick/prevention.html.
821 39. Organization, W. H. *Weekly epidemiological update - 9 March 2021*; 2021.
822 40. Krzywinski, M.; Schein, J.; Birol, I.; Connors, J.; Gascoyne, R.; Horsman, D.; Jones, S.
823 J.; Marra, M. A., Circos: an information aesthetic for comparative genomics. *Genome Res* **2009,**
824 *19* (9), 1639-45.
825 41. Greenhalgh, T.; Jimenez, J. L.; Prather, K. A.; Tufekci, Z.; Fisman, D.; Schooley, R., Ten
826 scientific reasons in support of airborne transmission of SARS-CoV-2. *Lancet* **2021**.
827 42. Richard, M.; Kok, A.; de Meulder, D.; Bestebroer, T. M.; Lamers, M. M.; Okba, N. M.
828 A.; Fentener van Vlissingen, M.; Rockx, B.; Haagmans, B. L.; Koopmans, M. P. G.; Fouchier, R.
829 A. M.; Herfst, S., SARS-CoV-2 is transmitted via contact and via the air between ferrets. *Nat*
830 *Commun* **2020,** *11* (1), 3496.
831 43. Sia, S. F.; Yan, L. M.; Chin, A. W. H.; Fung, K.; Choy, K. T.; Wong, A. Y. L.;
832 Kaewpreedee, P.; Perera, R. A. P. M.; Poon, L. L. M.; Nicholls, J. M.; Peiris, M.; Yen, H. L.,
833 Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* **2020,** *583* (7818),
834 834-838.

44. Bao, L.; Gao, H.; Deng, W.; Lv, Q.; Yu, H.; Liu, M.; Yu, P.; Liu, J.; Qu, Y.; Gong, S.; Lin, K.; Qi, F.; Xu, Y.; Li, F.; Xiao, C.; Xue, J.; Song, Z.; Xiang, Z.; Wang, G.; Wang, S.; Liu, X.; Zhao, W.; Han, Y.; Wei, Q.; Qin, C., Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 via Close Contact and Respiratory Droplets Among Human Angiotensin-Converting Enzyme 2 Mice. *J Infect Dis* **2020,** *222* (4), 551-555.

45. Yan, J.; Grantham, M.; Pantelic, J.; Bueno de Mesquita, P. J.; Albert, B.; Liu, F.; Ehrman, S.; Milton, D. K.; Consortium, E., Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. *Proc Natl Acad Sci U S A* **2018,** *115* (5), 1081-1086.

46. Lindsley, W. G.; Noti, J. D.; Blachere, F. M.; Thewlis, R. E.; Martin, S. B.; Othumpangat, S.; Noorbakhsh, B.; Goldsmith, W. T.; Vishnu, A.; Palmer, J. E.; Clark, K. E.; Beezhold, D. H., Viable influenza A virus in airborne particles from human coughs. *J Occup Environ Hyg* **2015,** *12* (2), 107-13.

47. Kulkarni, H.; Smith, C. M.; Lee, D. o. H.; Hirst, R. A.; Easton, A. J.; O'Callaghan, C., Evidence of Respiratory Syncytial Virus Spread by Aerosol. Time to Revisit Infection Control Strategies? *Am J Respir Crit Care Med* **2016,** *194* (3), 308-16.

48. Yu, I. T.; Li, Y.; Wong, T. W.; Tam, W.; Chan, A. T.; Lee, J. H.; Leung, D. Y.; Ho, T., Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med* **2004,** *350* (17), 1731-9.

49. Abrahão, J. S.; Sacchetto, L.; Rezende, I. M.; Rodrigues, R. A. L.; Crispim, A. P. C.; Moura, C.; Mendonça, D. C.; Reis, E.; Souza, F.; Oliveira, G. F. G.; Domingos, I.; de Miranda Boratto, P. V.; Silva, P. H. B.; Queiroz, V. F.; Machado, T. B.; Andrade, L. A. F.; Lourenço, K. L.; Silva, T.; Oliveira, G. P.; de Souza Alves, V.; Alves, P. A.; Kroon, E. G.; de Souza Trindade, G.; Drumond, B. P., Detection of SARS-CoV-2 RNA on public surfaces in a densely populated urban area of Brazil: A potential tool for monitoring the circulation of infected patients. *Sci Total Environ* **2021,** *766*, 142645.

50. Biryukov, J.; Boydston, J. A.; Dunning, R. A.; Yeager, J. J.; Wood, S.; Reese, A. L.; Ferris, A.; Miller, D.; Weaver, W.; Zeitouni, N. E.; Phillips, A.; Freeburger, D.; Hooper, I.; Ratnesar-Shumate, S.; Yolitz, J.; Krause, M.; Williams, G.; Dawson, D. G.; Herzog, A.; Dabisch, P.; Wahl, V.; Hevey, M. C.; Altamura, L. A., Increasing Temperature and Relative Humidity Accelerates Inactivation of SARS-CoV-2 on Surfaces. *mSphere* **2020,** *5* (4).

51. Qi, H.; Xiao, S.; Shi, R.; Ward, M. P.; Chen, Y.; Tu, W.; Su, Q.; Wang, W.; Wang, X.; Zhang, Z., COVID-19 transmission in Mainland China is associated with temperature and humidity: A time-series analysis. *Sci Total Environ* **2020,** *728*, 138778.

52. Harvey, A. P.; Fuhrmeister, E. R.; Cantrell, M.; Pitol, A. K.; Swarthout, J. M.; Powers, J. E.; Nadimpalli, M. L.; Julian, T. R.; Pickering, A. J., Longitudinal monitoring of SARS-CoV-2 RNA on high-touch surfaces in a community setting. *medRxiv* **2020**.

53. Luo, L.; Liu, D.; Zhang, H.; Li, Z.; Zhen, R.; Zhang, X.; Xie, H.; Song, W.; Liu, J.; Huang, Q.; Yang, X.; Chen, Z.; Mao, C., Air and surface contamination in non-health care settings among 641 environmental specimens of 39 COVID-19 cases. *PLoS Negl Trop Dis* **2020,** *14* (10), e0008570.

54. Cai, J.; Sun, W.; Huang, J.; Gamber, M.; Wu, J.; He, G., Indirect Virus Transmission in Cluster of COVID-19 Cases, Wenzhou, China, 2020. *Emerg Infect Dis* **2020,** *26* (6), 1343-1345.

55. Di Carlo, P.; Chiacchiaretta, P.; Sinjari, B.; Aruffo, E.; Stuppia, L.; De Laurenzi, V.; Di Tomo, P.; Pelusi, L.; Potenza, F.; Veronese, A.; Vecchiet, J.; Falasca, K.; Ucciferri, C., Air and surface measurements of SARS-CoV-2 inside a bus during normal operation. *PLoS One* **2020,** *15* (11), e0235943.

56. Mondelli, M. U.; Colaneri, M.; Seminari, E. M.; Baldanti, F.; Bruno, R., Low risk of SARS-CoV-2 transmission by fomites in real-life conditions. *Lancet Infect Dis* **2020**.

57. Kim, Y. I.; Kim, S. G.; Kim, S. M.; Kim, E. H.; Park, S. J.; Yu, K. M.; Chang, J. H.; Kim, E. J.; Lee, S.; Casel, M. A. B.; Um, J.; Song, M. S.; Jeong, H. W.; Lai, V. D.; Kim, Y.; Chin, B. S.; Park, J. S.; Chung, K. H.; Foo, S. S.; Poo, H.; Mo, I. P.; Lee, O. J.; Webby, R. J.; Jung, J. U.;

887 Choi, Y. K., Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. *Cell Host Microbe* **2020,**
888 *27* (5), 704-709.e2.
889 58. Azene, Z. N.; Merid, M. W.; Muluneh, A. G.; Geberu, D. M.; Kassa, G. M.; Yenit, M.
890 K.; Tilahun, S. Y.; Gelaye, K. A.; Mekonnen, H. S.; Azagew, A. W.; Wubneh, C. A.; Belay, G.
891 M.; Asmamaw, N. T.; Agegnehu, C. D.; Azale, T.; Tamiru, A. T.; Rade, B. K.; Taye, E. B.;
892 Taddese, A. A.; Andualem, Z.; Dagne, H.; Gashaye, K. T.; Kabito, G. G.; Mekonnen, T. H.;
893 Daba, S.; Azanaw, J.; Adane, T.; Alemayeyu, M., Adherence towards COVID-19 mitigation
894 measures and its associated factors among Gondar City residents: A community-based cross-
895 sectional study in Northwest Ethiopia. *PLoS One* **2020,** *15* (12), e0244265.
896 59. Azlan, A. A.; Hamzah, M. R.; Sern, T. J.; Ayub, S. H.; Mohamad, E., Public knowledge,
897 attitudes and practices towards COVID-19: A cross-sectional study in Malaysia. *PLoS One* **2020,**
898 *15* (5), e0233668.
899 60. Shewasinad Yehualashet, S.; Asefa, K. K.; Mekonnen, A. G.; Gemeda, B. N.; Shiferaw,
900 W. S.; Aynalem, Y. A.; Bilchut, A. H.; Derseh, B. T.; Mekuria, A. D.; Mekonnen, W. N.;
901 Meseret, W. A.; Tegegnework, S. S.; Abosetegn, A. E., Predictors of adherence to COVID-19
902 prevention measure among communities in North Shoa Zone, Ethiopia based on health belief
903 model: A cross-sectional study. *PLoS One* **2021,** *16* (1), e0246006.

904