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Detection of SARS-CoV-2 on surfaces of an Intensive Care Unit

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Abstract— *The study aimed to evaluate the presence of SARS-CoV-2 on surfaces in an Intensive Care Unit and to identify the role these surfaces may play in the transmission of this virus. The research was exploratory, descriptive, prospective, experimental research, with quantitative approach, conducted in the second half of 2020 in a public hospital of reference in the care for critically ill patients with COVID-19 in Santarém-Pará.It comprised 45 samples of surfaces of the most touched areas in the adult ITU of patients diagnosed with COVID-19 through RT-PCR: corresponding to 9 (nine) samples for each of the surfaces (on the mattress; side rails and bed control panel; mechanical ventilator circuit; bedside table and infusion pump). The samples were collected during the day in an occupied bed, using a swab on the extension of the surface. In the sequence they were submitted to the extraction process of genetic material, Ribonucleic Acid (RNA), of the SARS-CoV-2 virus, and later submitted to the amplification of the genetic material.According to the data obtained, all the surface samples analyzed in the present study tested negative for SARS-CoV-2, considering that the analysis was performed in ITU, in the period when inpatients were infected with SARS-CoV-2 and in transmissibility phase. These results suggest that the risk of exposure to a contaminated surface within an ITU is low, provided that preventive measures and sanitization routines are maintained.Cleaning and disinfection of care surfaces, using the standard protocol recommended by the National Health Surveillance Agency - Anvisa, are considered effective measures in containing SARS-CoV-2 transmission and infectivity.*

I. INTRODUCTION

The pandemic caused by the new coronavirus SARS-CoV-2 has been growing exponentially in the world and has reinforced the interest for the *Coronaviridae* family, more specifically the human coronaviruses (HCoV), historically known for causing respiratory tract infections, requiring admission to Intensive Care Units (ICU) for more severe respiratory problems, motivating several institutions to carry out research and monitoring of COVID-19. [1,2]

In this sense, human-to-human transmission occurs in the incubation period between 02 to 14 days, facilitating its spread by droplets, hands or contaminated surfaces of

therapy units. [3] These units are inevitably a large reservoir of opportunistic pathogens, so that healthcare-associated infections (HAIs) can be acquired not only by patients who present higher susceptibility, but also by visitors and employees of the hospital itself. [4] Moreover, these viruses show an environmental resistance that increases the possibility of transference between contaminated hosts, through surfaces, hands, among others. This resistance has led to the need to develop efficient prevention measures in order to reduce the viral load. As there are still no treatments, and the possibility of genetic mutation of the virus that could reduce the effectiveness of currently available vaccines cannot be ruled out, it is necessary to use other approaches to manage the infection and especially its prevention.[5-7]

The control of pathogen spread in the hospital environment is a practice based on cleaning and disinfection of contaminated surfaces, which are mandatory requirements for managers and healthcare workers. Commonly to this, chemicals traditionally, such as sodium hypochlorite at 0.1%, alcohol at 62 to 71% and hydrogen peroxide 0.5% have been used for surface disinfection in terminal or concurrent cleaning in hospital settings. [8,9]

Due to the significant increase in cases and the occupation of almost all available beds in both the public and private network, the need to verify the environment in which patients are receiving care has arisen. From this perspective, the objective of this study was to evaluate the presence of SARS-CoV-2 viral RNA on frequently manipulated surfaces in an ICU of a public hospital of medium and high complexity in the interior of the Amazon, and to identify the role that these surfaces can play in the transmission of this virus.

II. METHODS

1.1 Design, study site and period

Exploratory, descriptive, prospective, experimental research, with a quantitative approach, carried out at the RegionalHospital of Baixo Amazonas (HRBA), a public health unit of medium and high complexity that serves users of the Unified Health System (SUS). It is currently run by the Social Organization Pró-Saúde Associação Beneficente de Assistência Social e Hospitalar. This hospital is part of the flow of care for severe patients with COVID-19 in Santarém-Pará.

1.2 Sample acquisition and conditioning protocol

The sample composition was chosen according to the high frequency in which the surfaces are manipulated during the care and stay of the infected patient, obtained before and after concurrent disinfection, as described below: 70% alcohol was sprayed on the areas of interest (mattress; lateral grid and bed control panel; mechanical ventilator circuit; bedside table and infusion pump) and removed after sixty seconds of contact with the surface, using disposable multi-purpose wipes (Perfex ®).

The samples were identified with codes A1 to A5 (Fig. 1-5). A region of interest was defined on each surface, depending on the manipulation site. Each sample was collected in triplicate, generating subcodes that corresponded to the collection of the same surface within the same group. The information regarding each code, such as collection day, time, bed, surface, tested region, and sample number was catalogued and stored for later correlation.

To standardize the samples, the collection period between the fifth and seventh day of the appearance of the first symptoms of the flu syndrome was eligible, in individuals with positive PCR (Polymerase Chain Reaction) results for Covid-19.

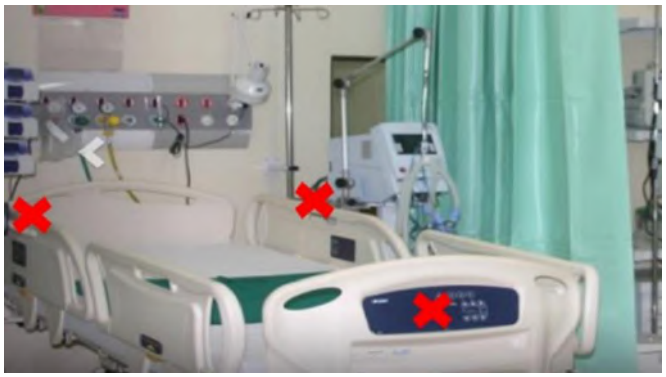

Fig.1. samplecollection sites - (A1) side rails and bed panel.

Fig.2. (A2) bed.

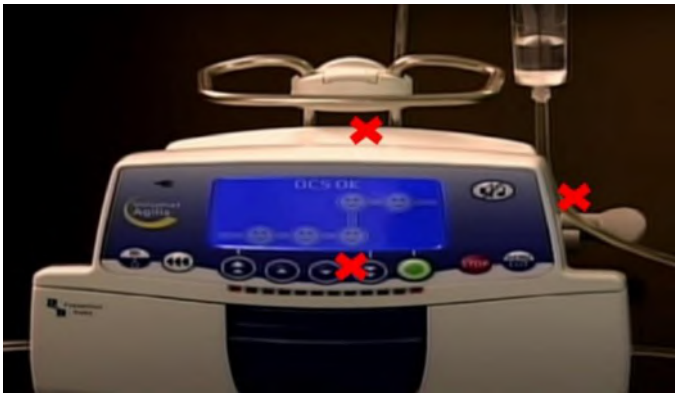

Fig.3. (A3) infusion pump.

Fig.4. (A4) bedside table.

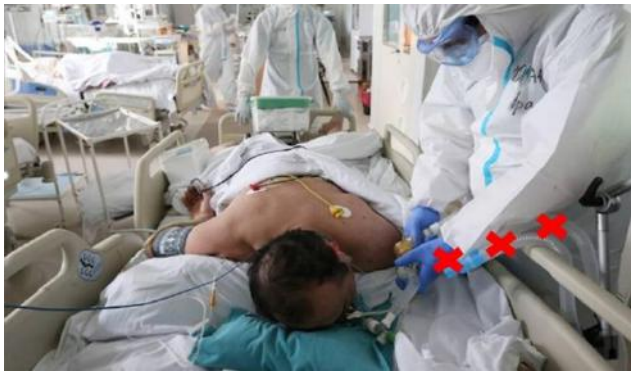

Fig.5. (A5) mechanical ventilator circuit.

The data collection procedure comprised 45 samples in total and was carried out in two phases: a) obtaining samples from the most manipulated areas of the care units in the ICU of the HRBA (mattress; lateral grid and bed control panel; mechanical ventilator circuit; bedside table, and infusion pump) and b) testing the samples for the presence of viral RNA in the Center for Diagnostic Medicine - CDM1.

The samples were collected by zig-zag swabbing the surface with a sterile Rayon swab, from left to right, at 5 mm intervals, diagonally, to standardize the material quantification process. The collection area was delimited at 100 cm^2 (approximate area 10x10 cm).

Each swab was placed in a collection tube containing 0.9% NaCl, labeled and stored at -20°C. Subsequently, the samples were placed in a thermal box with reusable ice at a temperature between 2°C and 8°C, on a support wrapped with cotton and absorbent blanket for greater security and stability in transport to the analysis laboratory, where molecular analysis of the samples was performed.

The samples of the surfaces were collected after the concurrent cleaning, which was performed with the disinfectant agent 70% alcohol, [10] sprayed all over the surfaces (mattress; lateral grid and bed control panel; mechanical ventilator circuit; bedside table and infusion pump) and removed by mechanical friction after sixty seconds of contact with the surface, using disposable multi-purpose cloths (Perfex ®), a procedure performed by a nursing technician with exclusive function of concurrent cleaning at each shift and/or need.

In cases of spillage of organic matter, which may prevent direct contact with the disinfectant on the surface and inactivate its germicidal properties, [11] the technique used continued to be removal of the excess with a paper towel, then sprayed the solution of VIREX® (Sodium Hypochlorite 1%) on the site, after 5 minutes it is removed with the help of disposable multi-purpose wipes (Perfex ®), according to the Work Instruction (IT) of the HRBA.

It is noteworthy that the processes of cleaning and disinfection of surfaces of the care units of the adult ICU of the HRBA, underwent several changes according to the legislation in force at the time (ANVISA, 2020). [12]

1.3 Detection and analysis of SARS-CoV-2 by means of RT-PCR

In an accredited laboratory for molecular analysis, the samples were submitted to the process of extraction of genetic material, Ribonucleic Acid (RNA), from the SARS-CoV-2 virus. For viral RNA extraction, 200µL of an aliquot from each sample was used and extracted according to the protocol defined by the supplier of the BIOPUR Kit Mini Spin Virus DNA/RNA 2.0 kit from Mobius Life Science, adapted with the addition of the ethanol drying step for 30 seconds at 15,000 RPM (Rotation Per Minute) before the dilution step.

After extraction, the samples were subjected to amplification of the SARS-CoV-2 virus genetic material. Cycling, mix preparation, detection markers, and extraction quality control protocols were followed according to the guidelines in the Mobius Life Science XGEN Master COVID-19 kit. RT-qPCR (Reverse Transcription Followed by Real-Time Polymerase Chain Reaction) reactions were performed on the QIAGEN Rotor Gene 5plex HRM equipment. The threshold value for all virus detection markers (N and ORF1ab genes) and extraction quality control was considered to be in the range of 0.03 to 0.3.

For the validation of the assay parameters the amplification value Ct (Cycle Threshold) of the positive

control and null for the extraction quality control was considered valid. For the virus detection markers, it was considered ≥ 18 ≤ 38, while for the negative control the amplification signal in the extraction quality control and the Ct value was null or > 38 in the virus detection markers (Gene N and Gene ORF 1ab) (Table 1).

Table 1. Parameters for assay validation.

	Valid Assay		**Non-valid Assay**	
	Positive Control	Negative Control	Positive Control	Negative Control
	Ct Value	Ct Value	Ct Value	Ct Value
Extraction quality control	Null	Presence of amplification signal	Presence of amplification signal	Null
Gene N	≥ 18 ≤ 38	Null or> 38	Null or> 38	≥ 18 ≤ 38
Gene ORF1ab	≥ 18 ≤ 38	Null or> 38	Null or> 38	≥ 18 ≤ 38

CT - amplification value.

III. RESULTS

The sampling comprised 45 surface samples from most touched areas in the adult ICU of patients diagnosed with COVID-19 through RT-PCR. All analyzed samples tested negative for COVID-19 RNA (Table 2), it should be noted that the samples were collected from ICU beds with patients who were infected and in the transmissibility phase. These results suggest that the risk of exposure to a contaminated surface within an ICU is low, provided that preventive measures and sanitization routines are maintained.

Table 2. Collections obtained by RT-PCR for screening of SARS-CoV-2 RNA.

Surface Places	**No. of tests**	**Result**
A1	09	Not detected
A2	09	Not detected
A3	09	Not detected
A4	09	Not detected
A5	09	Not detected
TOTAL	**45 tests**	

IV. DISCUSSION

The absence of detection of SARS-CoV-2 by RT-PCR in the surfaces of the adult ICU of the HRBA suggests that disinfection of surfaces is a clearly indicated and effective practice in the prevention and transmission of this pathogen because it is a single-stranded enveloped virus, i.e., it has a lipoprotein structure around the genomic RNA, facilitating its inactivation by several sanitizing products. In this sense, the survival of this microorganism in inanimate places has become a challenge for effective cleaning of healthcare environments, hence the urgent need to develop means to make the environment safe, not only for patients but also for professionals working in nosocomial environments. [13] On the other hand, because it is an extremely infectious virus, it requires surface disinfection methods that use effective products. Among the various sanitizers used in surface disinfection, with potential inactivation potential of SARSCoV-2, we highlight alcohol 70%, quaternary ammonium salts, hydrogen peroxide and sodium hypochlorite.

Considering the ability of SARS-CoV-2 to survive on inanimate surfaces, and focusing on the confrontation of the new pandemic, the prevention of further spread becomes decisive for the success in reducing the ongoing outbreak, which has victimized about 22.1 million infected people in Brazil, 616 thousand dead, computing a mortality rate of 3.0%. In Santarém-Pará, until this last balance sheet there

were 611 thousand confirmed cases and 16,942 thousand deaths. [3,14]

The mode of Covid-19 transmission occurs mainly via respiratory droplets after coughing or sneezing from persons infected with SARS-CoV-2. [15] There is also the likelihood of autoinoculation of the virus after contact with contaminated surfaces. Thus, the ability of SARS-CoV2 to infect, as well as the extent and speed of it viral spread, depend on the characteristics of both the virus and the surfaces on which it is found. [9,15,17]

A study conducted in the Republic of Korea, by Lee and collaborators(2020), corroborates with our findings, where RT-PCR was used for viral identification, 68 samples of surfaces from six hospitals, which had been cleaned and disinfected, there was no detection of SARS-CoV-2. However, two samples of the 12 that were collected at a rehabilitation center confirmed positive results for the presence of RNA polymerase and envelope genes of SARS-CoV-2,[16] indicating that cleaning procedures are effective in controlling SARS-CoV-2 infections.

The study by Chia and collaborators (2020) in Singapore evaluated 245 samples from the surfaces of 15 patients infected with COVID-19 in different stages of the disease, where high contamination was found in 66.7% of the patients evaluated in the first week of infection and 20% after the second week, with the highest rate of contamination on the floor (65%), the exhaust fan (60%), the bed rail (59%), and the bedside cabinet (47%), respectively, [18] results that refute our findings.

Added to the above, RT-PCR is considered a variable method among different biological samples to identify SARS-CoV-2. However, the diagnostic efficiency of several commercialized real-time RT-PCR kits for SARS-CoV-2 may be lower than ideal, i.e., <100%. [19] A study in China, for example, concluded that 41% of false negative RT-PCR test results remained false negative for a two-week period.[20] This fact is detrimental to the containment of the pandemic, since these individuals continue to transmit the disease because they are unaware of it.

It is important to point out that 45 samples were collected from 5 areas, corresponding to 100 cm² for each collection, at an ambient temperature that ranged from 18°C to 23°C, and an average relative humidity of 51%. In this sense, the survival of SARS-CoV-2 on surfaces may be influenced by environmental temperature, air humidity, and also by the viral load of the disease. [3,9] Despite all controversy regarding the survival time of CoVs on surfaces, it has been estimated that temperatures above 30 °C may reduce virus survival. [21,22]

Moreover, another point to be considered, are the high standards of hygiene and disinfection adopted by managers and hospital infection control service in the researched institution, which was recognized as one of the ten best public hospitals in Brazil in 2019 and qualified with the highest national certification, accredited with Excellence, granted by the National Accreditation Organization (ONA) which, since then advances in care quality.

V. CONCLUSION

The coronavirus pandemic brought to the scientific community, as well as to the patients, health team and laboratories, the challenge of facing an invisible and extremely powerful adversary, leading us to review some cares related to sanitation in care environments.

The absence of SARS-CoV-2 viral RNA on surfaces in the intensive care unit of the HRBA, suggests that surface disinfection is considered effective in containing transmission, infectivity of COVID-19, and maintaining a safe nosocomial environment.

It is noteworthy that the protocols for sanitization of the environment of the care units practiced by the HRBA are in line with the resolutions and determinations of the National Health Surveillance Agency (ANVISA) and the World Health Organization (WHO), which support good practices in health services. Finally, concurrent cleaning and disinfection of surfaces are considered essential elements for the control of healthcare-associated infections by ensuring an environment with clean surfaces, which may justify the absence of Covid-19 RNA detection on the surfaces evaluated.

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