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Surveillance of SARS-CoV-2 and nosocomial infections causing agents in healthcare settings

Detección de SARS-CoV-2 y agentes causantes de infecciones nosocomiales en instalaciones hospitalarias

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ABSTRACT

SARS-CoV-2 has affected millions of people around the world and resulted in millions of hospitalizations, where it represents a potential source of nosocomial infections for medical staff and patients and the spread of the virus. This study focused on the detection of SARS-CoV-2 as well as *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Staphylococcus aureus* in healthcare settings, including intensive care units, pediatric intensive care units, and COVID-19 care units. The results showed that SARS-CoV-2 was present in surface samples from the COVID-19 care unit, which detected the presence of the Delta variant. Furthermore, results showed the presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* on hospital surfaces. The presence of SARS-CoV-2 and nosocomial bacteria highlights the importance of constant microbiological monitoring to prevent co-infections and the occurrence of nosocomial infections during hospitalization.

KEY WORDS: SARS-CoV-2, nosocomial infections, Hospital, *Staphylococcus*, *Pseudomonas*.

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RESUMEN

El virus SARS-CoV-2 ha afectado a millones de personas en todo el mundo y ha provocado millones de hospitalizaciones, lo que representa una fuente potencial de infecciones nosocomiales para el personal médico y los pacientes, así como de propagación del virus. Este estudio se centró en la detección del SARS-CoV-2, así como de *Pseudomonas aeruginosa*, *Acinetobacter baumannii* y *Staphylococcus aureus* en ambientes hospitalarios, incluidas las unidades de cuidados intensivos para adultos, unidades de cuidados intensivos pediátricos y unidades de cuidados COVID-19. Los resultados mostraron la presencia del virus SARS-CoV-2 en muestras de superficies de la unidad de cuidados de COVID-19, detectando la presencia de la variante Delta. Adicionalmente, los resultados mostraron la presencia de *Pseudomonas aeruginosa* y *Staphylococcus aureus* en las superficies del hospital. La presencia de SARS-CoV-2 y bacterias nosocomiales resaltan la importancia de la vigilancia microbiológica constante para prevenir las coinfecciones y la aparición de infecciones nosocomiales durante la hospitalización.

PALABRAS CLAVE: SARS-CoV-2, infecciones nosocomiales, Hospital, *Staphylococcus*, *Pseudomonas*.

Introduction

COVID-19, the disease caused by SARS-CoV-2 and declared a pandemic in 2020, has affected the health of millions of people around the world and caused 664,873,023 confirmed cases (WHO, 2023). The U.S. CDC reports 101,873,730 cases, some requiring hospitalization, representing 5,866,347 hospitalizations due to COVID-19 from August 1, 2020, to January 23, 2023. Data from Mexico reports 7,348,292 confirmed cases, 9.79 % of which required hospitalization. During hospitalization, patients are at risk of nosocomial infections (CDC, 2023; CONACYT, 2023); Bardi *et al*. (2021) found that 40.7 % of patients acquired infections caused by bacteria and fungi during intensive care unit stay, and the predominant microorganisms were Gram-positive bacteria (55 %). In this regard, several studies have demonstrated the presence of nosocomial infection-causing agents, such as *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *and Staphylococcus aureus*; such presence and resistance against antimicrobials e.g. trimethoprim-sulfamethoxazole, oxacillin, vancomycin, meropenem, colistin, ceftazidime, among others in the hospital environment may deal in potential risk for patients during hospitalization, particularly in intensive care units (Esfahani *et al*., 2017; Rhoden *et al*., 2021; Aiesh *et al*., 2023; Tarigan *et al*., 2023).

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These suggest that the presence of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are the most common agents of pneumonia among COVID-19 patients, which is highly alarming given the burden of COVID-19 and the complications for patients as a product of coinfection with this kind of microorganisms in intensive care units (Mohammadnejad *et al*., 2021).

The incidence of nosocomial infections associated with COVID-19 can vary depending on the study, both in percentage and in organ involvement. Cheng *et al*. (2020) reported a 14.62 % incidence of nosocomial bacterial infections in patients with COVID-19 in China, of which 38.71 % were lower respiratory tract related, suggesting a significant association with age. Another study in Singapore reported the association of COVID-19 with nosocomial urinary tract infections in patients during their stay in the intensive care unit (Ong *et al*., 2021). The presence of SARS-CoV-2 in the hospital environment has been widely studied worldwide to understand airborne transmission. These studies suggest that viral particles of SARS-CoV-2 could circulate in hospital environments via aerosols, potentially contributing to long-term virus transmission, which is necessary to carry out infection control and prevention measures in healthcare settings (De Sousa *et al*., 2021; Du *et al*., 2021; Linde *et al*., 2023). Moharir *et al*. (2022) have found the presence of SARS-CoV-2 in air and surface samples from rooms of COVID-19 patients, supporting airborne transmission as a potential risk for virus transmission and the risk of infection with SARS-CoV-2 address with the occurrence of nosocomial infections in COVID-19 patients and medical staff (Zhou *et al*., 2020). The study aimed to detect SARS-CoV-2 and nosocomial infections-related bacterial pathogens from a hospital environment during critical COVID-19 waves in Mexico.

Materials and Methods

The present study was performed in Ahome, Sinaloa, Mexico; sampling was focused on surfaces such as sinks and beds and ambient air from the intensive care unit (ICU), pediatric intensive care unit (PICU), and COVID-19 care unit. The sampling took place from November 2021 to March 2022 at intervals of 15 days for a total of 7 visits. Three air samples were collected at each visit, one in each site (ICU, PICU, and COVID-19 care unit) for 42,000 L sampled air in this study. On the other hand, during the same visits, two surface samples (bed and sink) were collected for a total of 42 surface samples.

Surface samples

Surface samples were collected using sterile sponge swabs (Whirl-Pak, Nasco, USA) pre-moistened with 10 mL of sterile phosphate buffer solution. Each surface was scrubbed with a sponge, focusing on covering the entire surface of handrails and sinks. After sampling, each sponge was adjusted to a volume of 25 mL with a sterile phosphate buffer solution for further microbiological analysis.

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Air samples

Air sampling was performed using the MD8 Airport air filter device (Sartorious, Germany) according to the manufacturer's instructions, designed for airborne pathogens and consisting of the use of gelatin membrane filter cartridges with a pore size of 3 µm and a diameter of 80 mm. The air sampler was programmed at 50 L min^{-1} for 40 min for a volume of 2,000 L. After collecting the air sample, each cartridge was removed and placed in a cooler box at refrigerated temperatures for transport to the laboratory. For sample preparation, each filter was processed according to the manufacturer's instructions; the gelatin matrix was removed and placed in a 50 mL conical tube supplemented with 3 mL of nuclease-free water and centrifuged at 3,000 g, followed by incubation with thermal shaking at 37 °C for 10 min to dissolve the filter. Five equidistant points were sampled in each room to ensure a homogeneous and representative sampling of the rooms, with the sampling time at each point evenly distributed.

Microbiological confirmation

Only surface samples were examined for microbiological analysis. This was carried out based on the international standard method ISO 4833-2:2013 for the detection of *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Acinetobacter baumannii* using selective culture media such as cetrimide-selective agar, salt and mannitol agar and MacConkey agar with 8 mg L^{-1} gentamicin supplement. Briefly, a 1 mL aliquot of the previously homogenized sample was processed to obtain decimal dilutions of 10^{-1} and 10^{-2}, which were used for the spreading plating technique and incubated at 36 °C for 24 h; in this regard, each sample and decimal dilution was spread per triplicate. Results were expressed as colony-forming units per surface area (CFU) (International Standard. ISO 4833-2:2013; NOM-210-SSA1-2014).

Nucleic acid extraction

For viral RNA extraction, an aliquot of 200 µL of surface and air samples was processed with the TACO automated nucleic acid system using magnetic beads (GENE Reach, Taiwan), using the manufacturer's recommended DNA/RNA extraction. For DNA extraction, presumptive CFU of *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Staphylococcus aureus,* were processed with Quick Extract solution (Lucigen, USA). Briefly, a colony-forming unit was placed in 100 µL of Quick Extract solution, followed by vortexing for 15 s, incubation at 65 °C for 6 min, shaking for 15 s, and heating at 98 °C for 2 min. The nucleic acids were stored at −20 °C until use.

PCR identification

Virus detection was carried out by real-time RT-PCR using the CoviFlu Kit multiplex (Genes2Life) for the nucleocapsid gene of SARS-CoV-2 according to the manufacturer's instructions, using the primers and probes specific for nucleocapsid gene of SARS-CoV-2 with a mixture of 14.5 µL CoviFlu Primer Mix, 5 µL Buffer 5X and 0.5 µL enzyme and 5 µL ARN elution, the amplification protocol was carried out in the CFX-96 instrument (BioRad, USA) under the following reaction conditions: 50 °C for 30 min, 95 °C for 5 min, followed of 45 cycles at 95 °C for

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15 s and 62 °C for 30 s. A sample was considered positive if the Ct of the samples was lower than 38, and both the positive and negative controls performed as expected, indicated as a Gaussian curve behavior for each control, and with Ct values lower than 38 cycles each. According to the manufacturer, the CoviFlu Kit multiplex has a detection limit of around 50 copies per reaction for SARS-CoV-2 viral RNA.

Presumptive CFU for *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Staphylococcus aureus* were confirmed by real-time PCR using the iTaq Universal SYBR Green Supermix Kit (Bio-Rad, USA) with a reaction mix as follows: iTaq Universal SYBR Green Supermix 1X, 0.1 µM of each primer and 3 µL of eluted DNA for a total of 10 µL. The primer and conditions used in this work for each bacterium were as follows: 5´-CTGGGTCGAAAGGTGGTTGTTATC-3´ and 5´-GCGGCTGGTGCGGCTGAGTC-3´ for *Pseudomonas aeruginosa* (Choi *et al*., 2013), the amplification conditions for *Pseudomonas aeruginosa*, an initial denaturation cycle at 95 °C for 3 min, 35 cycles at 9 5°C for 60 s, 63 °C for 30 s, and 63 °C and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min, with an amplicon size of 232 bp. For *Acinetobacter baumannii* (Chiang *et al*., 2011), 5′-CATTATCACGGTAATTAGTG-3′ and 5′-AGAGCACTGTGCACTTAAG-3′ primers were used, 94 °C for 5 min and 35 cycles of 94 °C for 60 s, 62 °C for 60 s and 72 °C for 60 s; and a final extension at 72 °C for 10 min with a product of 208 bp. Finally, for confirmation of *Staphylococcus aureus*, 5´-GCGATTGATGGTGATACGGTT-3´ and 5´ AGCCAAGCCTTGACGAACTAAAGC-3´ (Hamdan-Partida *et al*., 2015). The protocol for PCR consisted of 94 °C for 5 min, 10 cycles of 94 °C for 40 s, 68 °C for 40 s, and 72 °C for 1 min, followed by 25 cycles of 94 °C for 60 s, 58 °C for 60 s, 72 °C for 2 min and a final extension at 72 °C for 10 min, for a fragment size of 279 bp.

For each case, *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 9721 were used as positive controls, and each run had a no-template control (NTC). The results were visualized using the Bio-Rad CFX Maestro version 1.0 (4.0.2325.0418) package.

Sequencing of positive viral samples

Positive samples were sequenced using the Illumina MiniSeq platform (2 x 150 PB paired end protocol, 300 cycles). The genomic libraries were prepared with the Nextera XT Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions and quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The obtained sequences were filtered, assembled, and converted into FASTA format and analyzed according to the "Comprehensive workflow for detecting coronavirus using Illumina benchtop systems".

To determine the lineages of SARS-CoV-2 variants, the sequences, and their metadata were deposited in the GISAID (Global Initiative on Sharing All Influenza Data) database to ensure immediate public access.

Phylogenetic analyses

The phylogenetic tree was constructed with representative sequences retrieved from GISAID EPI_SET_240709vn, available at 10.55876/gis8.240709vn, collected in Sinaloa from 2021-11-04

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to 2022-03-28, including the sequences of this study. Multiple alignment was performed using the MAFFT algorithm version 7 (https://mafft.cbrc.jp), and the phylogenetic tree was constructed using FastTree (http://www.microbesonline.org/fasttree/) with default options.

Results and Discussions

Detection of SARS-CoV-2 in air samples

Results showed that SARS-CoV-2 was not detected in air samples from hospital environments, including the intensive care unit (ICU), pediatric intensive care unit (PICU), and COVID-19 care unit (CCU). Of a total of 20 samples analyzed, real-time RT-PCR revealed no evidence of the presence of viral RNA in the air samples. In this regard, visualization of the RT-PCR real-time curves revealed no evidence of amplifying the viruses in this study. However, observing the amplification behavior of the RP gene was possible, and it was consistently detected in the analyzed samples. This indicates that RNA extraction, retro transcription, and amplification processes were successful in all samples. This ensures that negative results indicate the absence of virus particles or viral RNA and not an improper RNA extraction process.

The results of this study differ from those of other studies reporting the presence of SARS-CoV-2 in air samples; de Sousa *et al*. (2021) detected SARS-CoV-2 in air samples from a hospital environment in Sweden and reported 42 and 45 % positive samples from patient rooms and adjacent anterooms, respectively. We suspect that the non-detection of SARS-CoV-2 in our study compared to Sousa *et al*. (2021) could be due to the air filtration equipment and the volume of air filtered. Nevertheless, there are also reliable studies that demonstrate the absence of SARS-CoV-2 in ambient air hospitals; according to Lane *et al*. (2021a), of 576 aerosol samples, none were positive for SARS-CoV-2, highlighting the importance of environmental controls such as ventilation, negative pressure systems, and cleaning, as well as the use of personal protective equipment, the prevention of which is crucial and to reduce viral transmission (Landoas *et al*., 2021).

Detection of SARS-CoV-2 in surfaces

Results for SARS-CoV-2 in surface samples showed the presence of viral RNA in 4 positive samples, indicating the presence of viral RNA in 10 % of samples during this study (Figure 1). Interestingly, in the monitored areas, all samples from the intensive care unit and pediatric intensive care unit were reported negative for the presence of SARS-CoV-2. On the other hand, the presence of positive samples was detected only in the COVID-19 care unit, especially in samples from bed handrails. Our results are consistent with those of Ye *et al*. (2020), who found that the area most contaminated with SARS-CoV-2 was the COVID-19 care unit, suggesting that the hospital environment could be a potential source of virus spread, even among medical staff, patients, and visitors.

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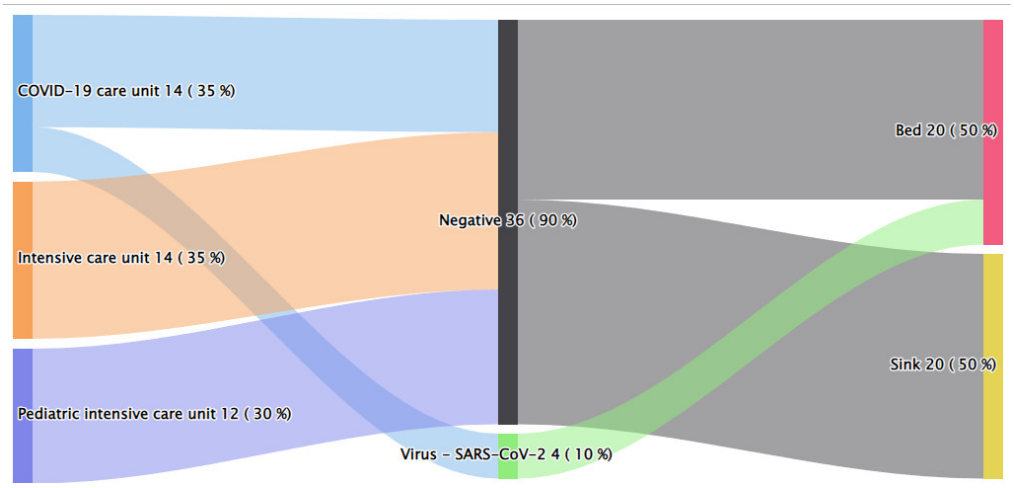

Figure 1. Distribution of SARS-CoV-2 positive samples in healthcare settings.

Sankey diagram of the distribution of negative and positive samples for SARS-CoV-2 in a hospital in Sinaloa from November 2021 to March 2022. The areas sampled were the COVID-19 care unit, the intensive care unit, and the pediatric intensive care unit.

The results of this study on the presence of SARS-CoV-2 in beds are consistent with previous studies such as Lane *et al*. (2021b), who detected the presence of SARS-CoV-2 in 19 out of 576 samples, particularly in the bed area, such as the head and foot areas of the bed. Pasquarella *et al*. (2020) demonstrated the presence of SARS-CoV-2 on hospital surfaces and found 26.6 % positive samples (4/15) from bed handrails, emergency buttons, handrails, and stethoscopes.

SARS-CoV-2 in beds is an alarming problem as previous results suggest that virus particles can survive in non-porous surfaces such as steel. In our study, the bed handrails were based on stainless steel. In this context, some studies reported the viability of SARS-CoV-2 in non-porous surfaces from 3.5 to 5.6 hours (Bonil *et al*., 2021). However, others suggest that viral particles can survive up to 72 hours (van Doremalen *et al*., 2020); such differences in survival can be attributed to the viral load in each study. In this context, based on the results of our study, it can be assumed that virus particles could be detectable for at least 14 days based on the sampling frequency in this study. In this regard, and based on results for clade grouping, support the idea that surrounding sequences share close-relatedness at the phylogenetic level, regardless of the time and source of isolation (Figure 2).

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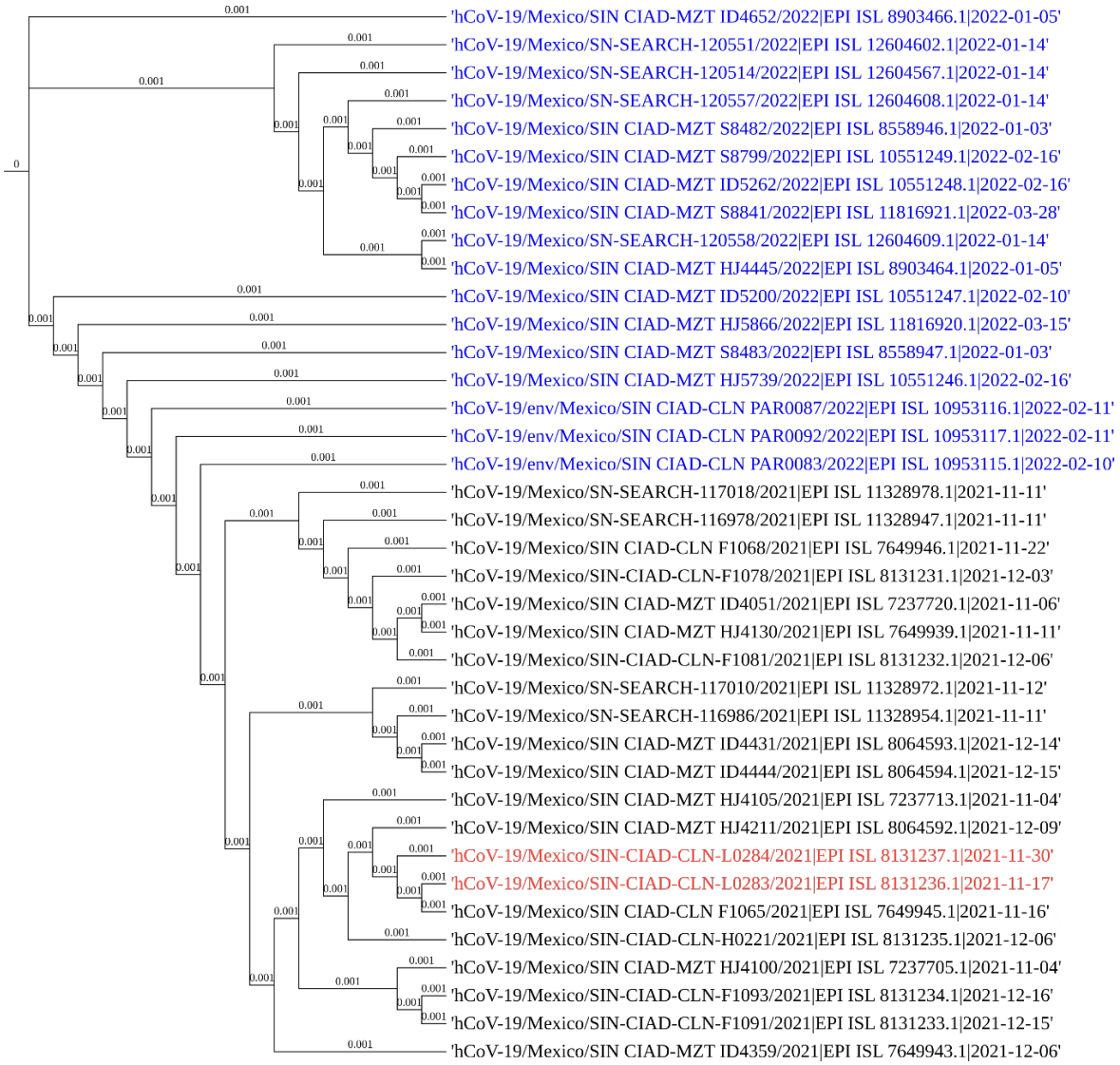

Figure 2. Phylogenetic tree of representative SARS-CoV-2 sequences from Sinaloa from November 2021 to March 2022.

Sequences representative of the main cities of Sinaloa were collected during the duration of this work from November 1, 2021, to March 31, 2022. Multiple alignment was performed using the MAFFT version 7 algorithm (https://mafft.cbrc.jp), and the phylogenetic tree was constructed using FastTree (http://www.microbesonline.org/fasttree/) with default options. Blue markers are sequences from 2022 samples, black markers are sequences from 2021 samples, and red markers are sequences from this study.

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Interestingly, those in the positive samples detected during Samples 1 and 2 in November 2021 belonged to the Delta variant. Results for SARS-CoV-2 variants in this study are consistent with the data from the National Consortium for Genome Surveillance (Co-Vi-Gen-Mex) in Mexico, as from September to November 2021, 99 % of the reported genomes belong to the Delta variant.

Detection of bacteria in surface samples

Of all samples, 82.5 % showed the presence of gram-positive bacteria, while 37.5 % showed the presence of gram-negative bacteria. Among the areas, the COVID-19 care unit was the most contaminated area during the study period, including the sink and bed railing (Figure 3).

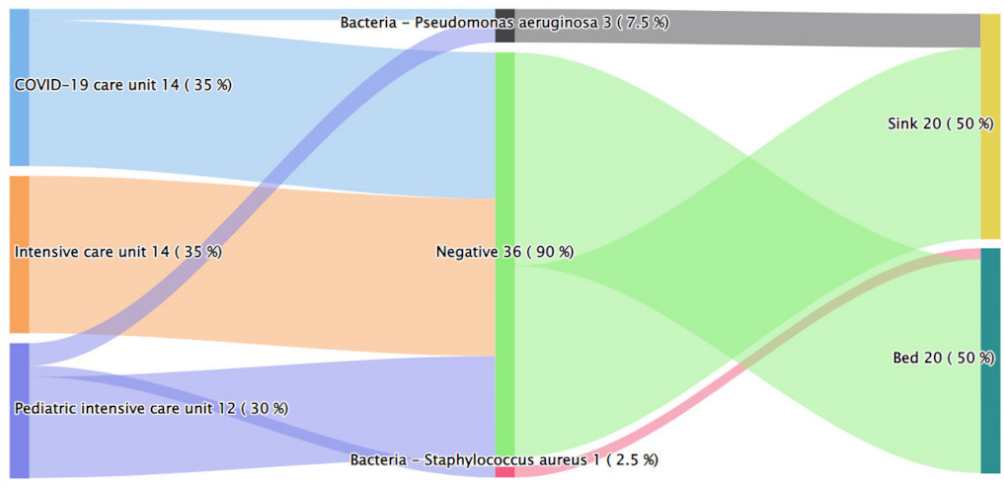

Figure 3. Presence and distribution of nosocomial bacteria on hospital surfaces.

Sankey plot of the distribution of negative and positive samples for *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a hospital in Sinaloa from November 2021 to March 2022. The areas sampled were the COVID-19 care unit, the intensive care unit, and the pediatric intensive care unit.

Pseudomonas aeruginosa

Bacterial isolates were subjected to PCR confirmation, which revealed the presence of *Pseudomonas aeruginosa* in three surface samples, detected in the first and third sampling in the pediatric intensive care unit and the COVID-19 care unit in the sink, with values up to 1,3 x10^3 CFU. The COVID-19 care unit showed the presence of *Pseudomonas aeruginosa* in a sink sample with a concentration of 270 CFU. According to our results, *Pseudomonas aeruginosa* was present

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in 7.5 % of samples, mainly isolated from sink samples (Figure 3). Our results are consistent with previous studies such as Olson *et al*., (1984), who reported that all hospital sinks and sewage were positive for *Pseudomonas aeruginosa*, with the same strains detected in patients, suggesting the possible transmission of nosocomial infections through cross-contamination. It is essential to consider that the presence of *Pseudomonas aeruginosa* in this environment can be favored by its survivability, which is reported to be able to survive on inanimate surfaces for between 6 hours and 16 months. This long-term survivability could contribute to *Pseudomonas aeruginosa* being one of the main causative agents of nosocomial pneumonia (Castañeda & Ordoñez, 2015; Paz *et al*., 2019).

Acinetobacter baumannii

PCR confirmation of bacterial isolates revealed the absence of *Acinetobacter baumannii* in all surface samples, including the intensive care unit, pediatric intensive care unit, and COVID-19 care unit. The results of our study are consistent with previous studies, such as those by D'Agata *et al*. (2000), who documented the absence of *Acinetobacter baumannii* in hospital environmental samples, regardless of the occurrence of infectious outbreaks. Nevertheless, some studies differ from our results. Ahmed *et al*. (2019) reported the presence of *Acinetobacter baumannii* in only 1.8 % of samples analyzed, suggesting a low incidence of this microorganism in hospitals in Egypt; Doscoph *et al*. (2019) reported the presence of *Acinetobacter baumannii*, which accounted for 46.7 % of the Gram-negative bacteria isolated in this study. Christoff *et al*. (2020) detected the presence of *Acinetobacter baumannii* in 7.39 % of samples out of 1,978 samples in a hospital in Brazil. Previous studies in Mexico have reported the presence of *Acinetobacter baumannii* in 8.7 % of surface samples from beds, tables, and medical equipment from two intermediate care units in a hospital in Guadalajara, Mexico (Arias-Flores et al., 2016). The absence of *Acinetobacter baumannii* in this study may be due to the appropriate use of disinfectants in healthcare settings, which may help reduce the abundance of this microorganism in such settings (Betchen *et al*., 2022).

Staphylococcus aureus

Staphylococcus aureus was confirmed in a bed rail sample from the pediatric intensive care unit, which had a concentration of 40 CFU per surface sample (Figure 3). This can be considered a low incidence of *Staphylococcus aureus* (2.5 %) in such settings, which differs from previous studies that reported incidences of up to 25 % of positive surface samples (Ahmed *et al*., 2019; Doscoph *et al*., 2019). The results of our study differ from the statistics of *Staphylococcus aureus* in hospitals, which, according to the literature, vary by 12-16 % in clinical samples from the USA, while the data for Mexico varies by 9.8 % (Sievert *et al*., 2012; Weiner *et al*., 2016; Arias *et al*., 2016). The importance of detecting *Staphylococcus aureus* in the hospital setting is due to its opportunistic and ubiquitous properties, namely the ability to survive on inanimate surfaces between 7 days and 7 months (Castañeda & Ordoñez, 2015). Another critical problem is antimicrobial resistance, which can complicate the selection of appropriate and effective antibiotics and harm the health of infected people.

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The low incidence and contamination rate of ambient air and surfaces can be attributed to good cleaning and disinfection practices by hospital staff, who can develop appropriate measures based on prescribed cleaning and disinfection practices, as well as the role in the use of disinfectant solutions to create resistant to prevent microorganisms. In this regard, previous studies reported that the incidence and distribution of nosocomial infection-causing agents such as *Staphylococcus aureus* and *Klebsiella pneumoniae* was higher before the pandemic event, contrarily to the pandemic event in different hospital departments (Su *et al*., 2021; Huang *et al*.,2023). This suggests creating a safe environment for patients and minimizing the likelihood of nosocomial infection during their hospitalization.

Conclusions

These study results suggest that the pandemic times have positively impacted cleaning and disinfection practices in healthcare, as evidenced by the low presence of bacteria with significant nosocomial relevance. It also suggests that the duration of residence of SARS-CoV-2 in the air is limited, so while airborne transmission via this route may not be the main route of spread for SARS-CoV-2 in healthcare settings, its prolonged presence on inanimate surfaces represents a potential risk to hospital staff and patients, which, in addition to bacterial contamination, may increase the likelihood of the occurrence of nosocomial infections. However, reinforcing best practices for mandatory cleaning and disinfection can further reduce the presence and risk of bacterial and viral pathogens spreading.

Author contribution

José Andrés Medrano-Félix. - Conceptualization and writing of the original draft, formal analysis, and figures. Juan Daniel Lira-Morales. - Methodology, formal analysis, and figures. Valeria Lizbeth Gurrola-López. - Methodology and formal analysis. Célida Isabel Martínez-Rodríguez. Cristóbal. - Supervision, writing, and editing. Nohelia Castro-del Campo. - Conceptualization, supervision, writing, and editing.

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Ethical declarations

Not applicable

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Declaration of informed consent

Not applicable

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Conflict of Interests

The authors declare no conflict of interest.

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