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Environmental biocontamination by SARS-CoV-2 Virus in the hospital setting

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ABSTRACT

Background: Demonstrating the capability to isolate biological material from the environment was fundamental to supporting any transmission route. Various and inconsistent methodologies have been used to address this issue; however, the debate in scientific societies about the possibility of airborne transmission as a source of SARS-CoV-2 spread remained open.
Objective: To analyze SARS-CoV-2 contamination in the air and on surfaces in a hospital setting during the COVID-19 pandemic.
Methods: This study involved air and surface sampling in the emergency, hospitalization, and intensive care unit areas of the Ramón y Cajal University Hospital. A consistent methodology was used for all samples, and clinical and environmental parameters and characterization of each location were recorded.
Results: A total of 234 samples were collected, comprising 160 surface samples and 74 air samples, of which 6.84 % tested positive (13/160 surface samples and 3/74 air samples). High-contact surfaces had the highest proportion of positive samples (12/13). All positive air samples were identified within 2 m of patients who had recently developed symptoms (<5 days). High dependency and elevated temperatures seemed to indicate a higher risk of environmental biocontamination. Additionally, there was a higher risk of contamination in the intensive care units than in the hospitalization or emergency units.

Background

In January 2020, the World Health Organization (WHO) declared a global public health emergency due to the outbreak of a new coronavirus (SARS-CoV-2). By the end of the first wave, 109 million cases were reported worldwide, with three million of these in Spain (COVID-19 Map 2024).

The first coronavirus that raised concerns due to sustained human-to-human transmission was SARS-CoV, which caused highly lethal pneumonia in 2003 .It was not limited to family or casual contact but significantly impacted the hospital environment. Its high transmission capacity raised questions about the role of droplet transmission, leading to new hypotheses on the potential role of airborne or contact transmission in the spread of respiratory viruses. However, the rapid containment of the outbreaks hindered the development of adequate research on this issue (Zhou et al., 2020).

During the SARS-CoV-2 pandemic, airborne transmission became a key focus in scientific discourse (Drossinos and Stilianakis, 2020).

Studies on viral persistence on surfaces conducted during the SARS-CoV epidemic were replicated (Kampf et al., 2020). Other researchers

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explored airborne transmission, yielding widely varying results. In China, (Cheng et al., 2020), found no positive airborne samples, while, (Liu et al., 2020), reported a 51 % positivity rate. Both studies used the same sampling device but with different designs and methodologies, which further complicates the comparison of results. The lack of standardised methods for sample collection is a significant challenge, highlighting the need for further research and collaboration in this area.

Despite a decrease in cases, the role of airborne transmission in the infection process remains unclear (Salzberger et al., 2021). The insufficient information published to date makes it difficult to determine the exact impact of different transmission routes on infection. Given the observed similarities with closely related respiratory viruses like SARS-CoV and MERS-CoV, studying the transmission routes in these viruses and understanding factors that determine which route predominates is crucial for effective epidemic control in the future.

This study aims to analyze the presence of SARS-CoV-2 in the air and on surfaces within facilities housing COVID-19 patients in a tertiary-level hospital, using a standardized and consistent methodology for all samples. It will also examine the correlation between patient-associated variables and the characteristics of the hospital wards.

Materials and methods

Study design and sample selection

A descriptive study was conducted from December 15, 2020, to April 14, 2021, and it was an 835-bed tertiary hospital.

Patients admitted to the Emergency Department (ED), Inpatient Wards (IW), and Intensive Care Unit (ICU) were selected for participation.

An opportunistic sampling method was used, selecting patients based on RT-PCR tests performed by the Microbiology Department within the last 24 h, with a cycle threshold (Ct) ≤30 were included. Due to workload and sampling time, four patients were selected per day for the study.

Sampling procedure

As detailed in Fig. 1, 2 air samples and 5 surface samples were collected in the patients' rooms.

Air samples for microbiological studies were obtained using a Sartorius MD 8 air sampler (Sartorius, Germany), which utilizes a gelatin membrane filtration method. This method draws in 1000 ml at a speed of 50 ml/min for 20 mins at a height of 1.5 m.

Surface microbiological samples were collected using swabs designed for viral DNA/RNA sampling (DNA/RNA SHIELD TM, Zymor Research Corp., USA), which were pre-moistened with phosphate-buffered saline solution. These samples do not require cold chain maintenance for transportation to the laboratory.

Levels of CO2 and temperature were monitored using the Chauvin Arnoux CA1510 indoor air quality meter (Chauvin Arnoux Metrix, France). A threshold value of 700 ppm was adopted as the limit for indoor air quality, and a temperature range of 19 °C to 24 °C was considered suitable.

The sampling duration time was 40 mins, and sampling was conducted aseptically to prevent contamination. Once collected, the sample was sent to the Microbiology Department for processing in <2 h.

Microbiological analysis

Air samples were processed immediately after collection. Gelatin membrane filters were dissolved in 1.7 ml of 0.2 % Tween through agitation at 220 rpm at 37 °C for 15 mins. Surface samples were processed without prior treatment.

Subsequently, 500 μL of each sample was used for genetic material extraction using the semiautomatic platform Easymag (Biomeriux, France) following the manufacturer's instructions. For air samples, triplicate processing was carried out to enhance efficiency. Detection of SARS-CoV-2 was performed by RT-PCR using TaqPath™ COVID-19 (Thermofisher), targeting three distinct targets of SARS-CoV-2: the nucleocapsid gene (N), the spike gene (S), and the ORF1ab gene.

A sample with a Ct ≤ 38 was considered positive.

Variables

Patients related factors included days since the onset of symptoms indicative of SARS-CoV-2 (fever, cough, dyspnea, anosmia, ageusia, muscle aches, diarrhoea, chest pain, or headache) (Subdirección General de Vigilancia en Salud Pública de la Comunidad de Madrid 2022), dependency evaluated using the Barthel scale with scores ≤35 indicating severe dependency; days since hospital admission, aerosol-generating procedures performed during sampling (endotracheal intubation,

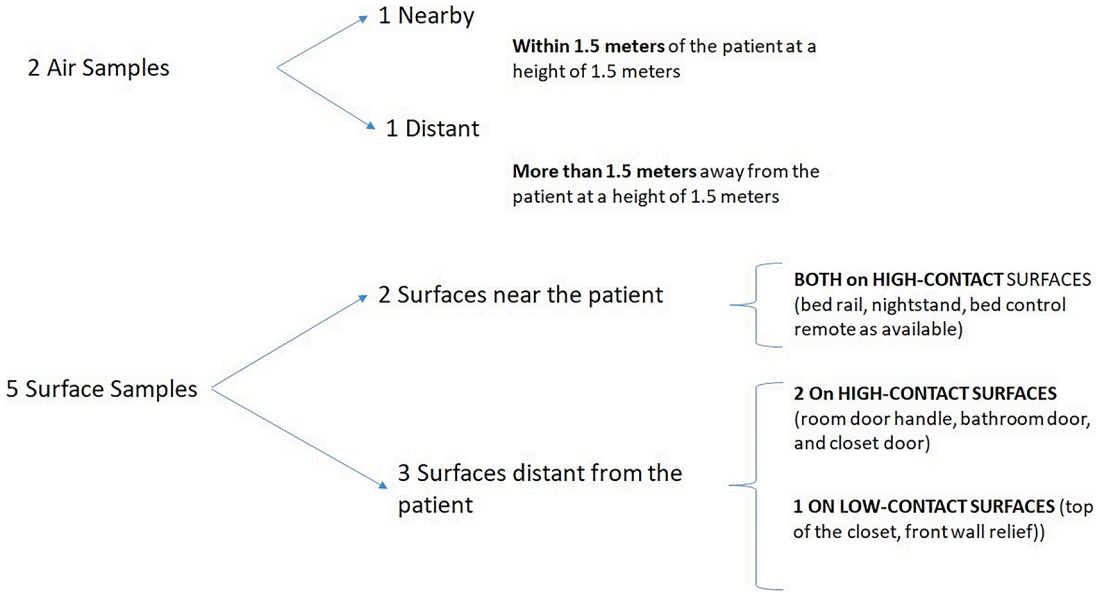

Fig. 1. Air and surface sampling scheme.

nebulisations).

Related to the ventilation system: type of air conditioning, temperature, and CO2 particle count.

Statistical analysis

All analyses were conducted using the R statistical program (The R Foundation). In the bivariate analysis, the $\chi 2$ test or Fisher's exact test was used for qualitative variables. For quantitative variables, the Student's *t*-test or Mann-Whitney U test was applied depending on whether the data followed a normal distribution. Results with p-values <0.05 were considered statistically significant. The ICU was taken as the control group for Odds ratio calculations.

Results

During the study period, 6132 patients with confirmed SARS-CoV-2 infection (RT-PCR positive) were admitted to the hospital.

A total of 37 COVID patients meeting the selection criteria were chosen: 22 patients were admitted to the ED, 12 to the IW, and 3 to the ICU.

The characteristics of the premises are detailed in Table 1.

A total of 234 samples were collected in patients' rooms or cubicles, 160 surfaces (68 %) and 74 air samples (32 %) in their rooms or cubicles.

Positive results were obtained in 16 samples (6.84 %), with 3 out of 74 being air samples (4.05 %) and 13 out of 160 being surface samples (8.13 %).

Three out of the 16 positive air samples (18.75 %), were from patients with symptom onset within 5 days, 2 of them were admitted to the ED and 1 IW. At the time of sampling all patients were within a distance of <2 m wearing masks. All three positive samples were obtained in rooms with temperatures > 24 °C. One of the positive samples was obtained after the performance of aerosol-generating procedures. All were at temperatures greater than 24 °C.

Of the positive surface samples, 13 out of 16 (81.25 %), were identified in patients who had shown symptom within the previous 5 days, this group included 6 patients admitted to the Emergency department, 5 ward, and 2 in the Intensive Care Unit. Among these positive samples, 7 out of 13 (53.84 %) were from surfaces close to the patient, while 6 out of 13 (46.15 %) were from more distant surfaces, including 1 low-contact surface. 10 out of 13 (76.92 %) were collected in environments where temperatures exceeded 24 °C.

The median time from symptom onset to sample collection was 5 days (4–14). 8 patients were admitted between 3 and 5 days before sampling, while 29 patients had been admitted >5 days prior.

21.79 % of the patients were dependent (Barthel ≤35). Of positive samples, 13.79 % were from rooms of dependent patients, compared to 4.5 % from rooms of non-dependent patients, showing significant differences between these groups ($p = 0.01$).

All samples were collected while the patients testing positive were present in the rooms or cubicles. Prior to starting the sample extraction, temperature and CO2 levels were recorded.

The median room temperature was 24.3 °C (23.6 °C-24.5 °C). Using 24 °C as the temperature threshold, 9.79 % of the positive samples were from rooms with temperatures ≥24 °C, and 2 % from rooms below this threshold, with these differences being statistically significant ($p < 0.01$).

The median CO2 value was 572 ppm (530–823), showing no variation with the type of air conditioning. 59.4 % of the measurements were below the indoor air quality threshold of 700 ppm.

All the characteristics are summarized in Table 2.

The Odds ratio, calculated with samples from the ED and IW as cases and those from the ICU as controls, was 0.66 (95 % CI 0.13–6.5). This indicates a 34 % increased risk of biocontamination in critical patient areas compared to the rest of the sampled units.

Discussion

Identification of the dominant mode of transmission is essential for developing appropriate and efficient strategies to control SARS-CoV-2 in hospitals. This could help in selecting suitable personal protective equipment and ventilation systems.

In our study, 37 locations were sampled, resulting in a total of 234 samples: 160 surface samples and 74 air samples. The positivity rates were 8.1 % for surface samples and 4.0 % for air samples. These data are

Table 1
Characterization of patient locations during sampling.

	Characteristics		
Location	Ventilation System	Door	Window
Intensive Care Unit	Triple-filter air conditioning with adjustable pressure 21 air changes /m^3	Single sliding access. Closed during sampling 33,3 %	Horizontal pivot
Ward	Conventional ventilation system 14 air changes/m^3	Single hinged access Closed during sampling 51,2 %	Horizontal pivot
Emergency Department	Conventional ventilation system 14 air changes/m^3	Sliding with multiple access	No window

Table 2
Description of PCR results in air and surface samples.

Collection Location	*N* = 234	PCR (+)		p	OR (IC 95 %)	
		N	%			
Emergency (ED)	126 (90|36) **	8 (6|2)**	6,35 %			
Hospitalization	87 (60|27) **	6 (5|1)**	6,90 %	s.n.s	0,66	0,13–6,5
Intensive Care Unit (ICU)	21 (15|6) **	2 (2|0)**	9,52 %			
Sample Type						
Surface – High contact	128	12	9,38 %	s.n.s	3,2	0,43–141,4
Surface -Low contact	32	1	3,13			
Air	74	3	4,05 %		2,09	0,54–11,7
Distance to Patient						
≤2 m	101	10	9,90 %	s.n.s	2,32	0,73–8,04
>2meters	133	6	4,51 %			
Environmental Parameters						
Temperature >24°*	143	14	9,80 %	0,03	4,82	1,06–44,58
Temperature ≤24°	91	2	2,20 %			
CO2≤700 ppm	158	12	7,59 %	s.n.s	0,67	0,15–2,33
CO2>700 ppm	76	4	5,26 %			
Door Open	182	13	7,14 %	s.n.s	0,79	0,14–3,06
Door Closed	52	3	5,77 %			
Clinical Data	*N* = 37	PCR (+)		p	OR	IC95 %
Symptom Onset		N	%			
≤5 days	17	6	35,29 %	s.n.s	1,19	0,37–3,8
>5 days	17	5	29,41 %			
Asymptomatic	3	1	33,33 %			
Dependency: Barthel ≤35						
Yes	9	6	66,67 %*	0,01	3,36	1,03–10,78
No	28	6	21,43 %			

Legend: *= statistically significant test. s.n.s= statistically not significant. **surface sample | airborne sample.

similar to those from a multicentre study conducted in 8 hospitals in UK (Moore et al., 2021), which analysed 334 samples from 44 patients, reporting a positivity rate of 8.9 % for surface samples and 7.3 % for air samples containing SARS-CoV-2 RNA (Ct threshold <34). These findings are consistent with early reviews, such as the one conducted by (Birgand et al., 2020), which reported an 8.3 % positivity rate for air samples.

There is no statistical evidence for this relationship, likely due to the small sample size in the study. However, the trend suggests that high-contact surfaces were more likely to yield positive samples, as only 1 out of the 32 samples taken from surfaces >2 m away – surfaces typically not manipulated by either the patient or personnel – tested positive.

No association was found between environmental contamination and days since symptom onset. The relationship between clinical phenomena and viral load remains unclear. Reviews such as (Comber et al., 2021) found no correlation between clinical severity and biocontamination. Similarly, (Zou et al., 2020), reported no differences in reported viral load between asymptomatic and symptomatic individuals within the first 5 days. Although there appears to be a tendency to consider this period as the most likely time for transmission, further exploration of this phenomenon is necessary.

Despite our wide confidence intervals, our results suggest an increased risk of environmental biocontamination in critical patient units. Similar observations have been made previously, with contamination rates reaching 86 % on surfaces such as stethoscopes and bed rails (Lucas et al., 2022). This may be related to other parameters we have discussed previously. Patients in these units undergo frequent handling and environmental manipulation during care activities. Nevertheless, further studies are essential to understand this association better and confirm it.

Fourteen out of sixteen positive results were recorded in rooms with temperatures above 24 °C. This finding contradicts the prediction made by mathematical models and the epidemiological patterns observed in Brazil at the start of the pandemic (Prata et al., 2020). Initially, it appeared that higher latitudes and colder temperatures might influence the spread, but as the pandemic evolved, a global distribution of cases was observed, regardless of temperature (Mandal and Panwar, 2020). At a microbiological level, it has been verified that temperatures between 24 and 25ºC represent the optimum point at which the virus maintains the highest stability in the environment (Rath and Kumar 2020). Current evidence suggests that the global spread of the virus is not solely dependent on low temperatures but may also relate to socio-sanitary conditions and the capacity for case detection (Salzberger et al., 2021). A reduction In viral survival is only at temperatures above 38ºC can we observe a decrease in its survival in the environment (Rath and Kumar 2020).

Significant differences were observed between the level of patient dependency and environmental contamination. A higher rate of positive samples was noted near dependent patients compared to independent ones. This correlation may be due to the greater environmental manipulation required for medication administration, hygiene, and maintaining protective measures such as proper mask placement in dependent patients.. This observation correlates with the distribution of high-contact surfaces identified. However, no studies have yet investigated this specific association between patient dependency and biocontamination.

One of the main strengths of this study is that, although we could not demonstrate sample viability through cell culture, relationships can still be established based on the Ct value from PCR testing and the potential for a positive viral culture. In our study, the Ct value was not arbitrarily chosen. Instead, it was based on a threshold that not only confirmed the positivity of the sample but also aligned with other studies that have demonstrated the viability of viral cultures based on a Ct parameter. In our case, this threshold was set at a Ct value of 20 for each patient. The relationship between Ct values and sample positivity provides a foundation for exploring this link further in future research, helping to address how viral load can influence the contamination of the surface (Singanayagam et al., 2020).

The period of our study marked the emergence of the first significant variant of SARS-CoV-2, identified as B.1.1.7 or Alpha. This occurred nearly a year after the initial outbreak of COVID-19. Despite the concerns regarding its potentially unique characteristics, our investigations revealed consistent behaviours with pre-existing strains. In our results, according to the temporal distribution of variants and the timing of extractions, we did not find differences in the positive results of the samplings. According to other studies, despite the rapid spread of the B.1.1.7 variant, there were no significant differences in hospitalization or mortality rates among the variants, supporting that the virus's behaviour in pathway transmission could not undergo radical changes with the emergence of the Alpha variant (Martínez-García et al., 2021). It is essential to recognize that traditional fluid dynamics in medicine, which categorized particles based on their size and dispersion capacity with potential transmission (Milton et al., 2013), has become outdated. Current understanding acknowledges that the fluid mass generated during activities, such as speaking or singing, can produce particles of various sizes, not merely droplets as previously assumed (World Health Organization 2024). Consequently, SARS-CoV-2 may serve as a catalyst for revising fluid dynamics concepts in medicine (Sanidad, 2020).

It is becoming increasingly evident that new factors potentially critical to airborne transmissions, such as humidity, electrostatic charges, or the impact of sunlight on particles' aerodynamic behaviour, must be considered (Gu et al., 2023). Therefore, it is crucial to continue enhancing data collection efforts to more definitively establish the influence of these factors.

As a limitation of our study, the number of samples was constrained by the availability of researchers due to the workload created by the pandemic. Additionally, studies on viral viability could not be conducted due to resource constraints.

This study provides evidence that proximity, environmental manipulation, and patient condition characteristics that require greater physical interaction with healthcare personnel, are highly relevant to the transmission phenomenon. Although airborne transmission is a plausible route for virus dissemination, droplets and direct contact continue to be key factors in the spread of infection.

It's important to emphasize that environmental sampling methodologies and methods for detecting the presence of the virus vary considerably. Consequently, significant uncertainties remain in estimating the risk of SARS-CoV-2 contamination on surfaces and in the air.

Conclusion

Environmental contamination has been detected more frequently in surface samples than in air samples within the hospital setting. Higher positivity rates have been observed on high-contact surfaces close to patient, particularly those with moderate to severe dependency. The detection of contamination in both air and surface samples highlights the need to consider both transmission pathways in hospital infection control, emphasizing contact and droplets as the primary route.

Credit author statement

Espejo Mambié, Moisés: Conception, design, data collection, analysis, interpretation of data, original drafting of the manuscript, and approval of final version.

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Galán Montemayor, Juan Carlos: Conception, data collection,

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Aranaz Andrés, Jesús María: Conception, design, interpretation of data, revised manuscript, and approval of final version.

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were carried out by Espejo-Mambié, M; San Jose-Saras, D; and Bischofberger C. Microbiological processing and analysis were performed by Galán, JC; Martínez-García, L; and Abreu, M. The initial manuscript draft was written by Espejo-Mambié, M, and all authors provided feedback on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Committee

The study was approved by the Ethics Committee of Ramón y Cajal University Hospital.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2025.100355.

Data availability

The datasets generated and/or analysed during the current study are not publicly available, but they are available from the corresponding author upon request.

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