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Environmental SARS-CoV-2 contamination in hospital rooms of patients with acute COVID-19

S. Nagle [a,b,c], Y. Tandjaoui-Lambiotte [b,c,d,e], M. Boubaya [f], G. Athenaïs [g], C. Alloui [g], C. Bloch-Queyrat [f], E. Carbonnelle [c,g], S. Brichler [g], Y. Cohen [b], J-R. Zahar [a,c,g,*], H. Delagrèverie [c,g]

[a] *Unité de Prévention du Risque Infectieux, Service de Microbiologie clinique, Groupe Hospitalier Paris Seine Saint-Denis, Université Sorbonne Paris Nord, Bobigny, France*
[b] *Réanimation Polyvalente, Groupe Hospitalier Paris Seine Saint-Denis, Université Sorbonne Paris Nord, Bobigny, France*
[c] *INSERM UMR 1137 IAME, Université de Paris and Université Sorbonne Paris Nord, Bobigny, France*
[d] *Pneumologie & Infectiologie, CH St Denis, Saint Denis, France*
[e] *INSERM UMR 1272 Hypoxie & Poumon, Bobigny, Université Sorbonne Paris Nord, Bobigny, France*
[f] *Département de Recherche Clinique, Groupe Hospitalier Paris Seine-Saint-Denis, Assistance Publique-Hôpitaux de Paris, Paris, France*
[g] *Unité de virologie, Service de Microbiologie Clinique, Groupe Hospitalier Paris Seine Saint-Denis, Université Sorbonne Paris Nord, Bobigny, France*

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SUMMARY

Objective: Data on the transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) remain conflicting. Airborne transmission is still debated. However, hospital risk control requires better understanding of the different modes of transmission. This study aimed to evaluate the frequency of, and factors associated with, environmental air and surface contamination in the rooms of patients with coronavirus disease 2019 in the acute phase of the disease.

Methods: Sixty-five consecutive patients were included in this study. For each patient, seven room surfaces, air 1 m and 3 m from the patient's head, the inner surface of the patient's mask, and the outer surface of healthcare workers' (HCW) masks were sampled. Environmental contamination was assessed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) for SARS-CoV-2 RNA on surfaces, air and masks. A viral isolation test was performed on Vero cells for samples with an RT-qPCR cycle threshold (Ct) $\leq$37.

Results: SARS-CoV-2 RNA was detected by RT-qPCR in 34%, 12%, 50% and 10% of surface, air, patient mask and HCW mask samples, respectively. Infectious virus was isolated in culture from two samples among the 85 positive samples with Ct $\leq$37. On multi-variate analysis, only a positive result for SARS-CoV-2 RT-qPCR for patients' face masks was found to be significantly associated with surface contamination (odds ratio 5.79, 95% confidence interval 1.31–25.67; P=0.025).

Conclusion: This study found that surface contamination by SARS-CoV-2 was more common than air and mask contamination. However, viable virus was rare. The inner surface of

* Corresponding author. Address: Unité de Prévention du Risque Infectieux, GH Paris Seine Saint-Denis, Université Sorbonne Paris Nord, Bobigny, France.
E-mail address: jeanralph.zahar@aphp.fr (J-R. Zahar).

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a patient's mask could be used as a marker to identify those at higher risk of contamination.

Introduction

In order to control the risk related to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection in healthcare facilities, it is essential to identify the different routes of transmission. While direct droplet transmission, requiring close contact (<1 m), is recognized [1,2], debate continues in the literature regarding the risk of airborne in-hospital transmission and indirect transmission through contact with surfaces contaminated by biological secretions. In a recent review of the literature [3], hospital airborne contamination was suggested by quantitative reverse transcription polymerase chain reaction (RT-qPCR) in 10% of air samples, but this was only confirmed by in-vitro culture in three of the 42 studies included. There are indirect arguments in favour of airborne transmission, notably the existence of proven long-distance contamination between two people [4] and the detection of SARS-CoV-2 RNA by RT-qPCR on air filters in hospital rooms [5]. Also, numerous contradictory studies have evaluated surface contamination due to SARS-CoV-2 in inpatient rooms, and reported that 4–80% of surfaces were contaminated [6–12].

Identifying the different modes of transmission can have major consequences for prevention, such as enhancing cleaning, adapting professional protective equipment and adding air cleaning treatment to protect healthcare workers.

The studies published to date have limitations; some did not consider the interval between symptom onset and the sampling date [6–9,12]. Most published studies were based on RT-qPCR results, and very few studies tried to evaluate the infectivity of SARS-CoV-2 detected on RT-qPCR. Further, no studies have been able to associate the existence of an infectious virus *in vitro* from RT-qPCR-positive surface samples [9] with the presence of a cytopathic effect after inoculation on Vero cells. Finally, studies identifying risk factors associated with environmental contamination are rare.

The primary objective of this study was estimation of the proportion of patients contaminating the air in their hospital room at 1 m distance. The secondary objectives were estimation of the proportion of patients contaminating the air in their hospital room at 3 m distance, the proportion of surfaces with viral contamination in the patient's room, and the proportion of masks worn by healthcare workers (HCW) that were contaminated on the external surface. In addition, risk factors associated with these situations were identified.

Methods

Patients

This prospective study was conducted from 22nd January to 8th April 2021 at Avicenna University Hospital, Assistance Publique – Hôpitaux de Paris, France. Consecutive adult patients, hospitalized in medical units during working hours, with positive nasopharyngeal swabs for SARS-CoV-2 RNA on RT-qPCR and symptoms of coronavirus disease 2019 for <15 days were included. Patients in the intensive care unit (ICU) were excluded.

The following data were collected: age, gender, active smoking, body mass index, medical history, and chronic diseases (e.g. hypertension, cardiovascular, neurovascular or chronic respiratory diseases, diabetes, active solid cancer, haematological malignancy, immunodepression). Clinical symptoms such as coughing, sneezing, fever, date of onset, and interval between symptom onset and sampling date were also recorded. Charlson Comorbidity Index (CCI), Katz Index of Independence in Activities of Daily Living (ADL Index), need for oxygen, aerosol treatment flow, vaccination against SARS-CoV-2, and interval between sampling and biocleaning (i.e. disinfection of patient's room) were also recorded. Laboratory data included date and result of SARS-CoV-2 RT-qPCR on nasopharyngeal swab collected as part of usual care.

Surface and air sampling

For each patient, SARS-CoV-2 RT-qPCR of air sampling of 600 L in 6 min 1 and 3 m distance from the patient's head, surface sampling and mask sampling (patient and HCWs) were performed on a given day (see online supplementary material).

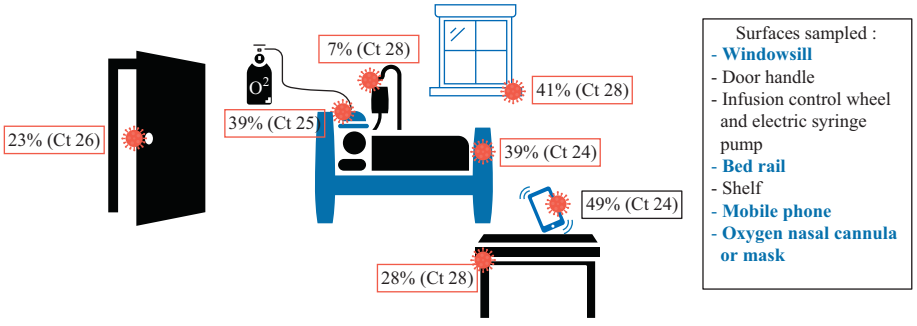

Figure 1. Frequency of positive surfaces by reverse transcription polymerase chain reaction (RT-PCR). ✹, each surface sampled for the study; %, percentage of samples contaminated with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA on each surface; Ct, median cycle threshold of positive SARS-CoV-2 quantitative RT-PCR on each surface. Blue surfaces represent the surfaces most frequently contaminated with SARS-CoV-2 RNA. This image was designed using the resources of Flaticon.com.

Seven surfaces were sampled in each patient's room (see online supplementary material (Figure 1)]: windowsill (>100 cm^2), inside door handles (entrance door and bathroom door), infusion control wheel and electric syringe pump, bed rails and remote controls for bed table adjustment, shelf (on 100 cm^2), patient's mobile phone, and patient's oxygen nasal cannula or oxygen mask. Finally, patients' surgical masks, worn for at least 15 min during sample collection, and HCWs' masks worn during care in a patient's room were collected (inner surface of patients' masks and external surface of HCWs' mask were sampled) and stored individually.

SARS-CoV-2 RT-qPCR and viral isolation test

For each sample, RT-qPCR was performed using m2000 Abbott Real Time Kit (Abbott, Chicago, IL, USA) targeting N and RdRp genes or SARS-COV-2 Real Star Altona Kit (Altona Diagnostics France, Joué-les-Tours, France) targeting S and E genes. All positive samples with a SARS-CoV-2 RNA RT-qPCR cycle threshold (Ct) ≤37 were tested for viral isolation (see online supplementary material). This threshold was chosen as this was the highest Ct associated with successful viral isolation on Vero cells described previously [13].

During the study period, the regular cleaning protocol for patients with SARS-CoV-2 infection was followed. Patients' rooms were cleaned daily using moistened microfibre cloths and disinfecting detergent solutions for bathrooms and the patient environment, and disposable cloths for floors.

This study was authorized by Ethics Committee CPP Sud-Mediterranée 2027 (Ref. 2020-A00897-32). No additional human samples were taken for the study. Information about the study and collection of each patient's consent to participate were given/taken orally.

Statistical analysis

Data collected were described using number and percentage for qualitative variables. Median and interquartile range (IQR) were used for quantitative variables. The proportion of patients who contaminated their surrounding environment (surface, air, patient's mask and HCW's mask) was estimated with 95% confidence intervals (CI) (Clopper—Pearson exact method).

Associations between contamination of the environment and patients' characteristics were analysed using Chi-squared test or Fisher's exact test for categorical variables, and Mann—Whitney test for quantitative variables. Contamination of surfaces and HCWs' masks were defined by at least one positive RT-qPCR result. The surfaces of patients' oxygen nasal cannulae or oxygen masks were not included in this analysis due to missing data. It was not possible to investigate risk factors associated with air contamination due to the low number of events. All factors with $P<0.20$ on univariate analysis of surface contamination were included in a multi-variate logistic regression. To account for missing data, the multivariate model was conducted using multiple imputations by chained equations with 10 imputations obtained after five iterations. The variables considered in the imputation models were all included in the univariate analyses. Results were aggregated by pooling the estimates obtained on each imputed dataset according to Rubin's rules.

The relationship between Ct for RT-qPCR SARS-CoV-2 on nasopharyngeal swab and the number of RT-qPCR-positive surfaces was evaluated using Spearman's rank correlation. All tests were two-sided, with $P<0.05$ considered to indicate significance. Analyses were performed using R Version 4.0.3.

Results

Population

Sixty-five patients hospitalized consecutively during the study period were included in this study. The characteristics of the study population are described in Table I. Among the study population, 46 (71%), six (9%) and nine (15%) patients were coughing or sneezing, had diarrhoea, or needed aerosol therapy on the day of sampling, respectively.

Environment samples

Room air samples were obtained for 59 patients. Seven (12%, 95% CI 4.9—22.9%) had at least one air sample positive for SARS-CoV-2 RNA on RT-qPCR. Median Ct was 38 (IQR 37—40) and 40 (IQR 39—42) 1 m and 3 m from the patient's head, respectively. Four (6.8%) patients had positive air samples 1 m from their head (4/59, 95% CI 1.88—16.46) and four patients (6.8%) had positive air samples 3 m from their head (4/59, 95% CI 1.88—16.46). It is important to note that only one patient had positive results for air samples taken both 1 m and 3 m from their head. Among the 392 surfaces swabbed, 134 (34%) were positive for SARS-CoV-2 RNA. The proportion of patients contaminating at least one of the seven surfaces was 64.6% (42/65, 95% CI 51.8—76.1%). Twenty-three patients (35.4%) had no contaminated surfaces, while all of the sampled surfaces were contaminated for 4.6% of patients. Positivity rates were highly variable, ranging from 7% for infusion control wheels and electric syringe pumps to 49% for patients' mobile phones, the most frequently contaminated surface (Figure 2). In each room, the median number of SARS-CoV-2-positive swabs was 2/7 (29%) (IQR 0—100%). The Ct of RT-qPCR for each surface was high, ranging from 34 to 38. Finally, among the seven patients with positive air samples, six also had a positive windowsill sample.

SARS-CoV-2 RNA was detected on the external surface of nine (10%) of the 90 face masks worn by HCWs in contact with 56 patients, and on the inner surface of 24 (50%) of the 48 masks worn by patients. Considering the masks worn by HCWs, nine (16%, 95% CI 7.6—28.3%) patients were the source of contamination, and all the RT-qPCR results had Ct >37. For the patients' masks, the median Ct was 35 (IQR 26—40).

Viral isolation

Among the 167 surface and mask samples with positive RT-qPCR results, viral isolation was attempted for 85 specimens (69 surfaces and 16 patients' masks) with Ct ≤37. The surfaces tested were: eight (9%) windowsills, nine (11%) door handles, one (1%) infusion control wheel, 14 (16%) bed rails, seven (8%) shelves, 19 (22%) mobile phones, and 11 (13%) oxygen nasal cannulae or masks. Figure 3 shows the flow chart for viral isolation tests. Correlation was noted between the number of positive surfaces and nasopharyngeal RT-qPCR Ct (): a low Ct was associated with a greater number of surfaces contaminated with SARS-CoV-2 RNA.

Table I
Characteristics of the population

Characteristics	All N=65	Surface contamination		P-value
		No N=23	Yes N=42	
Age (years)	75.0 (62.0–84.0)	69.0 (62.5–79.5)	76.0 (61.5–85.8)	0.31
Male sex	36 (55.4%)	12 (52.2%)	24 (57.1%)	0.90
Body mass index (kg/m^2)	28.1 (23.9–32.4)	27.6 (23.8–30.9)	28.3 (23.9–33.0)	0.64
Body mass index >30 kg/m^2	25 (43.9%)	7 (35.0%)	18 (48.6%)	0.48
Hypertension	39 (60.0%)	15 (65.2%)	24 (57.1%)	0.71
Cardiovascular (other than hypertension) or neurovascular disease	29 (44.6%)	12 (52.2%)	17 (40.5%)	0.52
Diabetes	21 (32.3%)	9 (39.1%)	12 (28.6%)	0.553
Chronic lung disease	12 (18.5%)	4 (17.4%)	8 (19.0%)	1
Active solid cancer	6 (9.2%)	1 (4.3%)	5 (11.9%)	0.41
Haematological malignancy	3 (4.6%)	0 (0.0%)	3 (7.1%)	0.55
Severe immunosuppression	4 (6.2%)	1 (4.3%)	3 (7.1%)	1
Active smoking	4 (6.8%)	2 (9.5%)	2 (5.3%)	0.61
Charlson Comorbidity Index	5.0 (3.0–7.0)	5.0 (3.0–6.0)	4.5 (3.0–7.0)	0.72
ADL Index	6.0 (5.5–6.0)	6.0 (6.0–6.0)	6.0 (4.5–6.0)	0.24
SARS-CoV-2 vaccination	4 (6.2%)	1 (4.3%)	3 (7.1%)	1
Symptomatic patient on day of sampling	63 (96.9%)	23 (100.0%)	40 (95.2%)	0.54
Interval between symptom onset and sampling date	6.0 (3.0–8.8)	8.0 (6.0–10.5)	5.0 (3.0–7.5)	0.025
At time of sampling				
Respiratory phenotype (cough, sneeze and/or need for oxygen)	58 (89.2%)	22 (95.7%)	36 (85.7%)	0.41
Cough and/or sneeze	46 (70.8%)	15 (65.2%)	31 (73.8%)	0.65
Need for oxygen	53 (81.5%)	21 (91.3%)	32 (76.2%)	0.19
Digestive phenotype (diarrhoea or vomiting)	6 (9.2%)	0 (0.0%)	6 (14.3%)	0.082
Fever (≥38 °C)	46 (70.8%)	16 (69.6%)	30 (71.4%)	1
48 h before sampling				
Respiratory phenotype (cough, sneeze and/or need for oxygen)	53 (81.5%)	20 (87.0%)	33 (78.6%)	0.52
Digestive phenotype (diarrhoea and/or vomiting)	3 (4.6%)	0 (0.0%)	3 (7.1%)	0.55
Fever (≥38 °C)	33 (50.8%)	11 (47.8%)	22 (52.4%)	0.93
WHO performance status ≥4[a]	30 (48.4%)	10 (50.0%)	20 (47.6%)	1
Death during hospitalization	21 (32.3%)	7 (30.4%)	14 (33.3%)	1
Biocleaning (days)	3.0 (1.0–4.0)	2.0 (1.0–4.0)	3.0 (2.0–4.2)	0.18
Biocleaning <24 h	15 (27.3%)	8 (34.8%)	7 (21.9%)	0.45
Ct of first nasopharyngeal SARS-CoV-2 RT-qPCR performed in hospital	18.5 (16.0–22.5)	22.0 (18.5–27.2)	17.0 (15.0–20.0)	0.0007
Ct of nasopharyngeal SARS-CoV-2 RT-qPCR closest to sampling date	19.0 (16.0–23.0)	23.0 (19.0–27.0)	17.0 (15.0–20.0)	0.0006
SARS-CoV-2 variant				0.19
Historical pandemic strain 20A D614G	35 (53.8%)	10 (43.5%)	25 (59.5%)	
Alpha variant (VOC 20I/501Y.V1)	18 (27.7%)	6 (26.1%)	12 (28.6%)	
Beta variant (VOC 20H/501Y.V2)	3 (4.6%)	1 (4.3%)	2 (4.8%)	
Unavailable	9 (13.8%)	6 (26.1%)	3 (7.1%)	

ADL, activities of daily living; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; WHO, World Health Organization; Ct, cycle threshold; RT-qPCR, quantitative reverse transcription polymerase chain reaction; VOC, variant of concern.
Data are presented as *N* (%) or median (interquartile range).
[a] WHO performance status for a patient (0, able to perform the same activity as before the disease; 1, decreased physical activity, but ambulatory and able to carry out work; 2, ambulatory and able to care for oneself, bedridden <50% of the time but unable to work; 3, able to perform only some activities, bedridden or in a chair >50% of the time; 4, unable to care for oneself, bedridden).

Viral growth was obtained from one surface sample (1/69, 1.5%): a swab from the oxygen nasal cannula of a patient who was sampled 3 days after symptom onset and presented Ct of 8 and 20 on the nasopharyngeal swab and nasal cannula, respectively.

Viral isolation was only successful for one patient's mask (in triplicate) out of 16 masks tested. RT-qPCR Ct was 37 for this patient, who was sampled 5 days after symptom onset and had a positive nasopharyngeal RT-qPCR with Ct of 16.

Of all the samples tested, in-vitro viral replication was obtained in two (2.3%) of 85 cases.

Risk factors associated with surface contamination: univariate analysis

On univariate analysis, surface contamination was associated with the interval between symptom onset and sampling

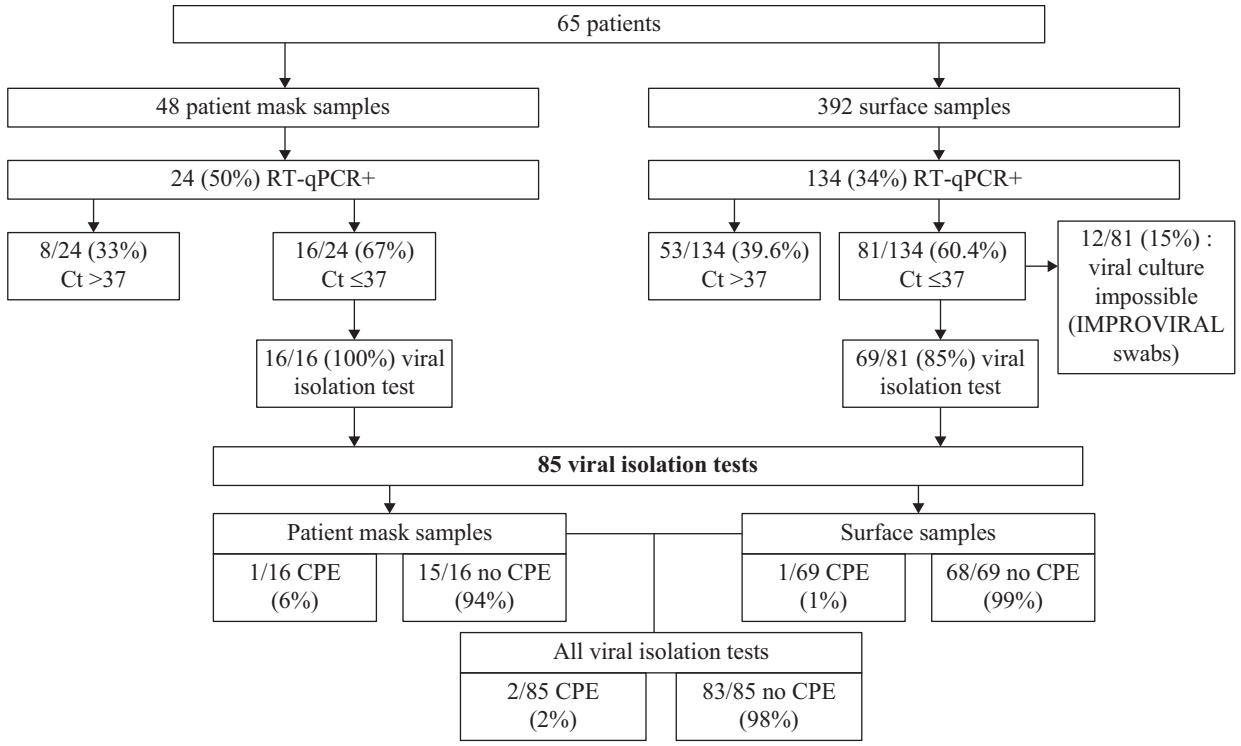

Figure 2. Flow chart for viral isolation tests. RT-qPCR, quantitative reverse transcription polymerase chain reaction; Ct, cycle threshold; CPE, cytopathic effect.

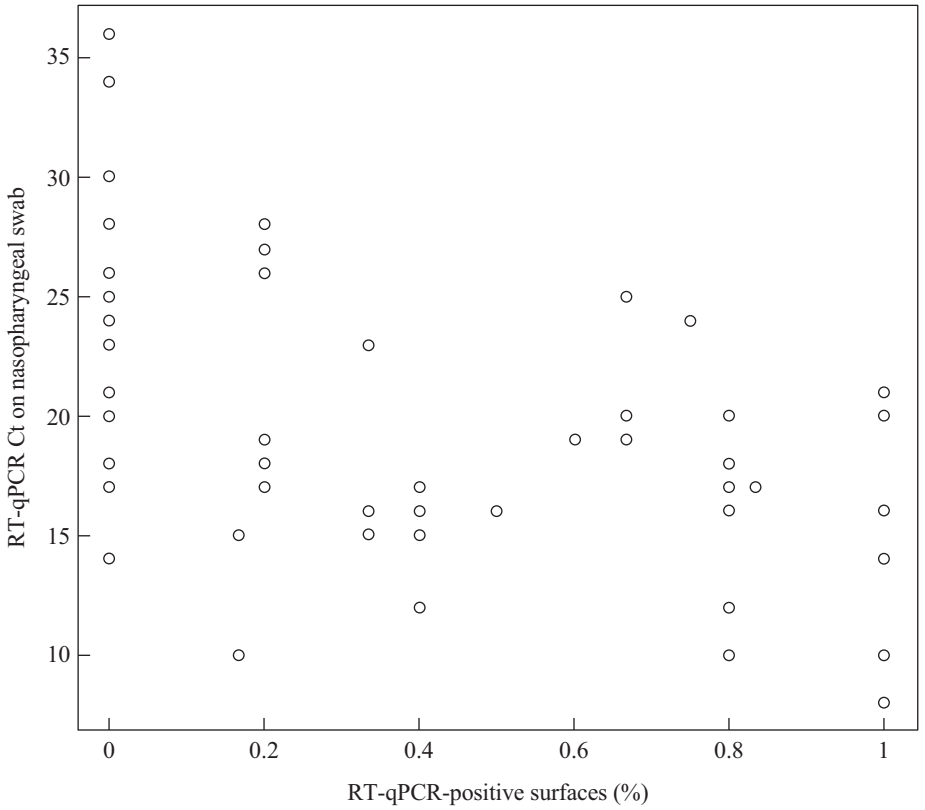

Figure 3. Correlation between cycle threshold (Ct) for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) quantitative reverse transcription polymerase chain reaction (RT-qPCR) on nasopharyngeal swab (test collected closest to sampling day) and the percentage of RT-qPCR-positive surfaces. Spearman's rank correlation coefficient of -0.48 ($P<0.001$).

date (P=0.025), Ct of the first nasopharyngeal SARS-CoV-2 RT-qPCR performed in hospital (P=0.001), Ct of nasopharyngeal SARS-CoV-2 RT-qPCR closest to the sampling date (P=0.001), and positivity of SARS-CoV-2 RT-qPCR on the inner surface of the patient's mask (P=0.016).

Contamination of patients' masks was only associated with a delay between symptom onset and sampling date (P=0.030) and Ct of the first nasopharyngeal SARS-CoV-2 RT-qPCR performed in hospital (P=0.021).

Contamination of HCWs' masks was not associated with any of the tested variables.

Multi-variate analysis of surface contamination showed that a positive result for SARS-CoV-2 RT-qPCR on the inner surface of patients' masks was a significant factor for surface contamination (OR 5.79, 95% CI 1.31–25.67; P=0.025), after adjustment for interval between symptom onset and sampling date (OR 0.95, 95% CI 0.78–1.16; P=0.63), Ct of nasopharyngeal SARS-CoV-2 RT-qPCR closest to the sampling date (OR 0.88, 95% CI 0.77–1.02; P=0.09) and biocleaning (OR 1.44, 95% CI 0.83–2.48; P=0.20).

Discussion

In this study, air contamination was less common than surface contamination around patients. Also, as suggested by several authors [6–12], despite the high frequency of positive samples on RT-qPCR, the viral isolation assay found few cases of viable virus.

These results are in agreement with several other studies suggesting that air contamination in hospitals reaches 10% of cases [3,12]. However, this remains the subject of debate, as higher RT-qPCR positivity rates in air, ranging from 20% (out of 44 air samples) to 100% (out of six air samples), have been reported [3,4,14]. These discrepancies can be explained by several factors that the authors have tried to consider in the present study. First, the risk could differ according to the interval between sampling and symptom onset [15]. In fact, numerous studies have included ventilated and non-ventilated ICU patients who were considered to be in an inflammatory and non-infectious phase of the disease [3], whereas the authors of the present study attempted to focus solely on patients in the acute phase of the disease. Second, the sampling method used could have had an impact on the result. In the present study, contrary to previous studies, the decision was made to sample a quantity that reflects the risk linked to a predefined duration of care. Also, in the present study, a quantity of air equivalent to the current volume inspired by a HCW in case of contact equivalent to 1 h of care was aspirated. Third, the present results were obtained using a very sensitive RT-qPCR, allowing the detection of 100 copies of virus/mL with sensitivity of 95%, and it was possible to detect any SARS-CoV-2 RNA concentration >2.3 viruses per L of air.

On univariate analysis, this study suggested correlation between environmental contamination and the interval between symptom onset and sampling date (Table I). Moreover, Spearman's rank correlation (Figure 2) was found between the nasopharyngeal Ct threshold and the percentage of RT-qPCR-positive surfaces (Figure 2). The data suggest correlation between viral load and environmental contamination. These results are in agreement with data published previously, with infectiousness correlated with the duration of evolution and the Ct threshold [15–24].

The authors were unable to conclude whether there is a specific mode of transmission concerning hospitalized patients. However, the data suggest that contact and droplet transmission play greater roles than airborne transmission. The low percentage of positive air samples compared with positive surface samples, and the fact that the most contaminated surfaces were those close to the patient, with the exception of the windowsill, was unexpected. However, the high frequency of windowsill contamination could suggest direct airborne contamination. Indeed, windowsills were located distant from the bed, so they could be considered as surfaces touched by patients or HCWs, and surfaces on which airborne viral particles may settle. The contamination measured could have been explained by either airborne contamination or indirect contamination. However, in a subgroup analysis (data not shown) comparing patients with contaminated windowsills with patients without contaminated windowsills, no difference was found in terms of dependence (ADL score, World Health Organization performance status) or symptoms (respiratory or digestive phenotype). The only difference noted was nasopharyngeal Ct at the time of sampling.

Surprisingly, only 50% of the masks collected from patients had positive SARS-CoV-2 RT-qPCR results (Figure 3), despite the fact that these patients were in the acute phase of the disease with positive nasopharyngeal RT-qPCR results; this led to discussion of the sensitivity of the technique used. However, these results are similar to those found in the literature. Indeed, in a study including 66 patients [25], SARS-CoV-2 RT-qPCR on two polyvinyl alcohol strips stuck to the inside of patients' masks, worn for 30 min, were only positive in 65–70% of cases, despite the fact that patients' nasopharyngeal RT-qPCR results were positive. Moreover, in the present study, the profile of patients with a positive mask on SARS-CoV-2 RT-qPCR was different from the profile of patients with a negative mask (shorter interval between symptom onset and sampling date, and lower Ct on nasopharyngeal swab).

A major strength of this study lies in the fact that it attempted to detect viable virus, unlike many other studies. Using a viral isolation model on Vero cells, only two environmental samples were found to be culture positive, suggesting that the viral inoculum identified in the samples was too low (Figure 3). However, it is important to note that, in accordance with other studies [13,16–24,26], positive samples with Ct >37 were excluded. Indeed, studies that sought to correlate Ct and the presence of viable virus suggested that no viral replication was detected when Ct was >24 [21] or >37 [13].

The present study tried to consider several factors that would have led to interpretation bias. In order to overcome the influence of cleaning, the multi-variate analysis was adjusted for time of completion. Nonetheless, it is important to note several limitations. Firstly, a specific air aspiration device was used, and other available devices [3] were not tested; as such, it cannot be confirmed that the device used has the best diagnostic performance. Secondly, there are several other confounding factors that are difficult to standardize in clinical practice, and which could have introduced interpretation bias. Several factors could modify the quality of air sampling, such as external conditions (e.g. humidity, temperature, dust, chemical composition of the air), air exchange and exterior wind conditions when opening windows. In this study, the patients were hospitalized in rooms without air treatment. The authors

tried to evaluate the effect of opening windows, but were unable to ensure the exhaustivity of the data. This could be a limitation because air flow dynamics, which are influenced by opening windows, are determinant for detection of a virus in the air and on surfaces in a patient's room, especially for remote and inaccessible surfaces. Thirdly, this study took place when SARS-CoV-2 variants 20A D614G (historical pandemic strain) and 20I/501Y.V1 (alpha variant) were the main viruses circulating at Avicena Hospital (54% and 28%, respectively). These viruses are no longer present today. Fourthly, it is important to moderate the conclusions as the duration of mask wearing was not standardized. Finally, this study was monocentric and included a small number of patients. In order to be representative, a larger study is needed.

To conclude, all of the results within the limits of the work performed and the population included suggest that the risk of droplet and contact transmission is greater than the risk of airborne transmission. Environmental contamination of patients' hospital rooms with SARS-CoV-2 appears to be frequent for surfaces and patients' masks, but at high Ct and without viral isolation on culture for 98% of samples tested. Air contamination and contamination of HCWs' masks are less common. On multi-variate analysis, surface contamination was associated with SARS-CoV-2 RT-qPCR positivity of the inner surface of patients' masks. The results of patients' mask samples could be used as a surrogate marker to identify those at higher risk of contamination, and to recommend intensified biocleaning in the rooms of these patients.

Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2022.05.003.

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