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Major Article

Positive no-touch surfaces and undetectable SARS-CoV-2 aerosols in long-term care facilities: An attempt to understand the contributing factors and the importance of timing in air sampling campaigns

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Background: Long-term care facilities (LTCF) are environments particularly favorable to coronavirus disease (SARS-CoV-2) pandemic outbreaks, due to the at-risk population they welcome and the close proximity of residents. Yet, the transmission dynamics of the disease in these establishments remain unclear.
Methods: Air and no-touch surfaces of 31 rooms from 7 LTCFs were sampled and SARS-CoV-2 was quantified by real-time reverse transcription polymerase chain reaction (RT-qPCR).
Results: Air samples were negative but viral genomes were recovered from 20 of 62 surface samples at concentrations ranging from 13 to 36,612 genomes/surface. Virus isolation (culture) from surface samples (n = 7) was negative.
Conclusions: The presence of viral RNA on no-touch surfaces is evidence of viral dissemination through air, but the lack of airborne viral particles in air samples suggests that they were not aerosolized in a significant manner during air sampling sessions. The air samples were collected 8 to 30 days after the residents' symptom onset, which could indicate that viruses are aerosolized early in the infection process. Additional research is needed to evaluate viral viability conservation and the potential role of direct contact and aerosols in SARS-CoV-2 transmission in these institutions.

BACKGROUND

Between December 2019 and January 2021, the coronavirus (COVID-19) pandemic caused by the Severe Acute Respiratory coronavirus type 2 (SARS-CoV-2) resulted in nearly 100 million confirmed cases and 2 million deaths.[1] People above 65 years old were 5-13 times more likely to be hospitalized and 90 to 630 times more likely to die from the disease than individuals between the age of 18 and 29 years old.[2] The oldest and most frail seniors require hours of daily assistance and many reside in long-term care facilities (LTCFs) where outbreaks of viral respiratory (influenza) and gastrointestinal

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tract infections (norovirus) are common[3]. SARS-CoV-2 transmission in LTCFs has also been reported worldwide, including in the United States,[4] the Netherlands[5] and Canada.[6] SARS-CoV-2 outbreaks are more likely to happen in confined/crowded congregate living spaces like LTCFs, nursing homes and prisons than traditional living spaces.[7,8] People living in LCTFs generally have limited mobility, live in close proximity to each other, and require close contact with care personnel, leading to increased number of potential transmission events.

Knowledge of SARS-CoV-2 spread is incomplete and it is still not clear how the virus is transmitted in LTCFs, particularly when recommended infection prevention strategies appear to be properly applied. Public health organizations recognized respiratory droplets and aerosols as major transmission routes for the virus.[9,10] Although SARS-CoV-2 virus preserves infectivity for days on various surfaces[11,12] and in the air,[13] no specific report clearly supports that COVID-19 can be transmitted via fomites, and it is not considered to be the main route of transmission.[14,15] On the other hand, it was suggested that aerosols (short or long distance transmission) could be involved in the transmission of COVID-19.[16-20] Indeed, it was reported that coughing, sneezing, talking or even breathing can lead to emission of SARS virus aerosols in both respirable and inhalable sizes.[21-23] Aerosols from various sizes (inhalable, thoracic and respirable) can be produced and enter the respiratory tract.[24-28] Since both SARS-CoV-1 and SARS-CoV-2 are phylogenetically highly similar, it seems possible that COVID-19 may also be spread by small particle aerosols.[29] Nonetheless, it is not clear how the emission of SARS-CoV-2 aerosols is modulated in both symptomatic and asymptomatic infected people. It seems that the earlier stages of COVID-19 are associated with emission rates as high as 10^5 viral RNA copies per min.[30]

Accurate information about airborne concentrations of SARS-CoV-2 is still sparse and no standardized or reference sampling and detection methods have been validated for this purpose.[31] Published reports have used various approaches of air and no-touch surface sampling but experimental parameters are sometimes ill-defined, such as particle sizes and concentrations, air sampling and downstream processing (type of sampler, sampled volume, nucleic acid purification, real-time reverse transcription polymerase chain reaction (RT-qPCR)), environmental parameters (high/low risk areas, air exchange rates, sampler position/location), and presence and type of aerosols generating procedures (AGP). In addition, the contribution to viral aerosolization of common interventions in LCTF such as the use of continuous positive airway pressure (CPAP) machines or toilet flushing were not described in the COVID literature, nor was the impact of poor ventilation. All these limitations and differences in experimental approaches complexify interpretation and make it difficult to generalize published knowledge to long-term-care facilities.[31] Nonetheless, these studies report positive air samples and no-touch surfaces in healthcare settings (≈8% to 100% positive),[30,32-37] suggesting SARS-CoV-2 may be aerosolized in COVID-19 LCTFs.

Other than control and prevention measures such as personal protective equipment and personal hygiene, appropriate ventilation should limit the spread of COVID-19.[38] de Man et al reported that inadequate ventilation in a nursing home building led to an outbreak that stemmed from aerosol transmission of SARS-CoV-2.[39] It was also stated in the press that broken ventilation (100% recirculation) could have allowed the aerosols to concentrate and spread in the building causing at least 200 cases and 64 deaths among the 236 residents.[40]

This prospective study was conducted to determine air and no-touch surface contamination by SARS-CoV-2 in LCTFs with COVID-19 outbreaks during spring 2020. Air and no-touch surface samples were simultaneously taken in COVID-19 positive patients' rooms. Contributing factors to the presence of SARS-CoV-2 aerosols in these healthcare settings were investigated and the outbreak calendar was obtained after the sampling visits. This paper adds knowledge that could help limit propagation of COVID-19 among resident and healthcare workers in LTCFs.

METHODS

Long-term care setting

Seven LTCFs in major cities of the province of Quebec were visited during spring 2020 on a convenience basis. The willingness of the establishments to allow sampling guided these choices. Rooms of patients diagnosed with COVID-19, placed under droplet/contact isolation precaution, and cohorted in a dedicated "red" zone, were sampled. These red zones were floors or wards hosting only positive residents. The LTCFs observed no visitors allowance policy as well as standard infection control practices. No significant aerosol mitigation measures were implemented (higher air exchange rate, negative pressure, etc.). Broad epidemiological data (number and proportion of infected individuals in time) was collected *a posteriori* from the overseeing provincial health organization (Supplementary Table 1). Since these are living environments, as opposed to hospitals and critical care settings, symptoms monitoring was not rigorous, and these data could not be reliably included in this article. Only the LTCFs II, III, and V had a central ventilation system with intake and extraction vents in the corridor, but not inside the rooms (Supplementary Table 1). The other LTFCs relied solely on passive ventilation. The windows inside the patients' rooms were not open during air sampling, but the door leading to the corridor had to be left open at the patients' discretion.

This experimental protocol was authorized by the ethical committee of Ste-Justine Hospital, project number MEO-21-2021-3475, through a multi-centered agreement.

A total of 93 samples from 31 rooms hosting a patient were included in this study. Three samples were collected simultaneously for each room, one air sample and two surface samples. Table 1 illustrates the number of rooms sampled for each LTCFs, as well as additional information regarding the number of days since diagnosis of the patient and the number of days since the first case was confirmed in the LTCF in comparison to the sampling date.

Air sampling

Air sampling was performed using an IOM Multidust sampler (SKC, Eighty Four, PA, USA) loaded with a 3 μm gelatin filter (Sartorius Stedim Biotech, Gottingen, Germany). The samplers were attached to a portable pump Gillian Air 5 (Gillian, Sensidyne, USA) and calibrated at 3 L/min. Sampling was performed for 4 hours (total volume of air = 720 L). They were put on furniture, at least 1.5m above the ground and placed approximately 2 m from the resident to limit the capture of droplets.

Filters were eluted on the day of sampling and stored at −80°C until RNA extraction. Gelatin filters were dropped in 900 μL of viral transport media (Redoxica, Little Rock, AR) at 37°C until complete dissolution (less than 5 minutes). The solution was divided in 400 μL aliquots and frozen immediately at −80°C.

Surface sampling

Two surfaces of approximately 10 cm^2 were sampled for each room using flocked swabs (Puritan, USA). Swabs were humected in 1 mL of viral transport media (Redoxica, Little Rock) prior to sampling. They were eluted in the remaining liquid after surface sampling. The swabbed surfaces were out of reach and unfrequently cleaned. Most swabs took a very dark color from the dust they collected. The elution liquid was divided in 400 μL aliquots and froze at −80°C until RNA extraction. The top of the door frame inside

Table 1
Quantification of surface swabs samples and related epidemiological data

Rooms ID	LTCF ID*	Active ventilation (air intake and extraction vent in the corridor)	Shelving unit swab sample (genomes equivalent/surface)†	Door frame swab sample quantification (genomes equivalent /surface)†	Number of days since diagnosis	Number of days since the first confirmed case in the LTCF
1	I-a	-	36,612	2,772	8	12
2	I-b		-	445	14	16
3	I-b		4,059	1,160	15	16
4	I-b		-	4,059	14	16
5	I-c		-	-	13	17
6	I-c		1,030	309	14	17
7	I-c		-	-	13	17
8	I-c		-	391	7	17
9	II	+	493	1,220	9	9
10	II		-	-	8	9
11	II		-	-	ND	9
12	II		-	-	9	9
13	III-a	+	-	-	27	48
14	III-b		-	-	22	46
15	IV	-	-	-	13	29
16	IV		-	-	15	29
17	IV		-	-	26	29
18	IV		-	-	30	29
19	V	+	-	-	13	35
20	V		2,440	-	23	35
21	V		-	-	12	35
22	V		328	-	27	35
23	VI	-	35	-	NA	
24	VI		-	51		
25	VI		-	11,890		
26	VI		13	-		
27	VI		2,616	-		
28	VII-a	-	75	-	NA	
29	VII-b		-	502	15	30
30	VII-c		-	-	25	30
31	VII-c		-	-	10	30

*Letters next to the LTCF ID indicate the different wards of an establishment.
†Negative results are considered to be below the limit of detection.

the room of the resident and the top of a shelving unit were sampled. These areas are located between 2 and 4 meters from resident if bedridden and are considered not touched or cleaned on a frequent basis, which would limit the inference of surface contamination by residents or workers and act as a proxy for viral propagation in the environment through air.

Sample RNA extraction and quantification

The sample treatment and quantification were described in Dumont-Leblond et al.[41] Briefly, the 400 μL aliquots of each type of sample was directly extracted using the MagMAX Viral RNA Isolation Kit (Applied Biosystems, Vilnius, Lithuania), following the manufacturer's instructions. The final elution volume was of 50 μL. Purified RNA was immediately quantified by RT-qPCR. Extraction controls (no template controls) were performed for each extraction batch.

SARS-CoV-2 quantifications were performed by RT-qPCR targeting the ORF1b of SARS-CoV-2. The Bio-Rad iTaq Universal Probes One-Step kit was used according to the manufacturer protocol (CA, USA). Logarithmic plasmid standard curves were used as positive controls (custom plasmid with the ORF1b insert) and to allow quantification. Results are expressed in equivalent genomes of SARS-CoV-2. Additional information regarding RT-qPCR reactions, probes, plasmids, and primers are available in the Supplementary Tables 2 and 3. Only RT-qPCR results under 40 Ct were considered positive.

Viral culture on swab samples with a positive RT-qPCR signals was attempted (both door and shelf samples from rooms 1 and 3, door sample from room 2, shelf samples from rooms 20 and 22). The method was described by Dumont-Leblond et al and is based on cytopathic effect detection with Vero E6 cells (African green monkey cells; American Type Culture Collection [ATCC]).

Statistical analysis

Nominal variables were expressed with frequencies and percentage (%) and were analyzed using Fisher's exact test. Continuous variables were reported as mean ±SD and analyzed using Student's t test. The normality assumption was verified with the Shapiro-Wilk tests on residuals from the statistical model. The Brown and Forsythe's variation of Levene's test statistic was used to verify the homogeneity of variances. A mixed model, looking into the number of genomic copies, was performed to compare the viral yield of sample swabs between shelving units and door frames. A first factor was linked to the comparison between doors and shelves and was analyzed as a repeated-measure factor with the use of a generalized covariance structure. The rooms were analyzed as a random factor. We used residual maximum likelihood as the method of estimation and the Kenward–Roger method to estimate denominator degrees of freedom for the tests of the fixed effect. The normality assumption was verified with the Shapiro-Wilk tests after a Cholesky factorization on residuals from the statistical model. The Brown and Forsythe's variation of Levene's test statistic was used to verify the homogeneity of variances. Statistical analyses were adjusted for the number of days since the diagnosis or the beginning of the outbreak. Data were log-transformed to respect these assumptions. The results were considered significant with P-values $< .05$. All analyses were conducted using the statistical package SAS v9.4 (SAS Institute Inc., Cary, NC).

RESULTS

SARS-CoV-2 RNA could not be detected in any of the air samples. Each essay was below the limit of detection of the method (>40 Ct).

In total, 20 of 62 swabs (32%) were positive for SARS-CoV-2 RNA by RT-qPCR. Ten swabs of each type of surface, from 16 rooms out of 31, were positive with a median concentration of 761.5 genome equivalents/surface (1st quartile = 74.6, 3rd quartile= 2,615.6) for shelving units and 830.8 genome equivalents/surface (1st quartile = 391.0, 3rd quartile= 2,772.0) for door frames. Positive swab samples for both types of surfaces were only found in rooms 1, 3, 6, and 9. No type of surface seems to allow for more frequent detection (Table 1). The viral load recovered from door frames and shelving units were not related (mixed model, $P = .7645$). Hence, one type of sample did not recover significantly more viral RNA. Viral culture was negative for all samples.

The presence of positive swab samples, independently of the type of surface, tends to be related to the absence of ventilation (Fisher's exact test, $P = .0515$). Positive swab samples seem to be more frequently found in LTCFs that relied only on passive ventilation (83.33% vs 16.67%). Only the LTCFs II, III, and V had active ventilation through the corridor (no air extraction directly in the rooms). Additional information regarding the LTCFs is available in Supplementary Table 1.

Rooms were sampled from 8 to 30 days after the patient was diagnosed and from 9 to 48 days since of the first confirmed case in the corresponding LCTF (Table 1) (see Supplementary Fig 1 for epidemiological curves). Establishing contact with LTCFs was challenging and their limited capability and willingness, at the time, to coordinate the arrival of our sampling team were restrictive factors to a more rapid deployment. The mean number of days since the patients' diagnosis or since the first confirmed case for rooms with positive surfaces, any surface, (15.1 and 21.9 days) or rooms without positive surfaces (16.8 and 26.2 days) were not significantly different (double-sided t test, $P = .7058$, .2539). The timing between sampling and the diagnosis date, or the first diagnosed case in the LTCF, did not explain why surfaces in some rooms were positive for SARS-CoV-2.

DISCUSSION

Viral RNA was recovered from 20 of 62 surface swabs from no-touch surfaces (door frames and shelving units). The viral load recovered (up to 36,612 genomes equivalent/surface) combined with the absence of direct contact on these surfaces could indicate that viruses were recurrently transported through air. Another study of LTCFs and health care settings has found the virus on no-touch, out of reach surfaces.[33] However, the impact of aerosolization and deposition on the viability of the virus is unsettled. We could not isolate the virus of our swab samples showing the highest virus load estimated by RT-qPCR.

Both types of sampled surfaces were positive 10 times at comparable concentration (mixed model, $P = .7645$) and double positive for only four rooms. Each type of surface sample detected the virus when the other could not (6/10), showing the importance of sampling various surfaces when investigating to presence of virus in the environment. The recovered quantity of viral material cannot be correlated to the total amount of viruses deposited since both time between deposition and sampling and viral RNA degradation dynamic are unknown.

The source of aerosolized particles that led to no-touch surfaces contamination could be the patients themselves through respiratory droplets generation. However, other mechanism may be involved such as aerosolization through fecal matter manipulation during diaper changes/flushing toilets.[42] In addition, virus found in settled dust on no-touch surfaces can be re-aerosolized in the environment and deposited elsewhere from a surface to another. Patient-emitted aerosols and re-aerosolized particles cannot be differentiated in this study.

When they are present, airborne particles can be inhaled and the virus can reach the respiratory tract.[43] SARS-CoV-2 could not be detected in any of the air samples. The residents' rooms were sampled from 8 to 30 days after they were first diagnosed with COVID-19. Evidence suggests that replication-competent virus in mild disease decreases after the symptom onset and that transmission happens more frequently within 5 days since the first symptoms.[44-49] Concentration of the virus RNA in the upper respiratory tract is also known to decline after symptoms onset.[48-52] The air sampling campaign might have missed the window of time in which aerosols were more highly present in the rooms due to stronger resident shedding. Since aerosolization from patients may mostly rely on sporadic events such as cough, the relatively short sampling time (4 hours) might have missed these events. Also, the possibility of underestimation caused by viral degradation during the sampling process cannot be completely discarded. However, a similar methodology was deployed in actively ventilated hospital rooms where airborne particles were detected.[41] A combination of poor timing and viral degradation may explain the lack of detection of aerosols in the presence of the patients, even in poorly ventilated rooms.

Currently no standardized protocols for the study of airborne SARS-CoV-2 have been proposed or validated, leading to limited ability to compare studies. The sample collection protocol in this work was guided by previous literature and expertise on the study of viral bioaerosols and on the very few published articles at the beginning of the pandemic.[37,41] To date, a consensus regarding a reproducible air sampling approach has not been reached even considering the various studies published to date.[31]

A weak relationship between positivity of samples, independently of the type of surface, and the absence of mechanical ventilation was found (double-sided t test, $P = .0515$), but did not reach significance. The presence of active ventilation, through the hallways air exchangers may have decreased the accumulation of virus on the surfaces by extracting aerosols before they could deposit. Other environmental factors (architectural, policies, staff, etc.) could vary between each LTFCs and may have influenced the observed relationship. Even if the relation between ventilation and aerosols or settled dust is weak, it is in accordance with the general belief that ventilation should reduce aerosols produced in confined areas. The limited number of samples collected, and the prospective nature of our study design, does not allow to draw strong conclusions about the role of deficient ventilation in those settings. Further work needs to be done to clearly illustrate the possible correlation.

This study provides very pertinent insight into the viral contamination of the environment in LTCFs. The combination of air and surface samples taken simultaneously adds temporal characterization to the contamination dynamics, as surfaces may be an adequate indicator of past aerosolization and deposition. The multicentric nature of this study also allows us to get a broader picture of possible viral transport in air since each establishment may slightly vary in terms of clientele, practices, and architectural conformation. However, these slight variations may obscure our ability to compare results. The difficulty to rapidly deploy sampling teams in this context of outbreaks constitutes a major limitation to this study, since they might have missed the pic level of viral aerosol production and exposition risks.

Epidemiological data reporting long-distance transmissions of COVID-19 have yet to be published. The level of contribution of the airborne route of transmission of SARS-CoV-2 is still to be defined and new models of a broader airborne model involving inhalable aerosols for SARS-CoV-2 transmission in low-risk health care settings is to be considered.[31] Cumulative data and positive air and no-touch surface samples found in healthcare facilities suggest that airborne transmission does not occur only for smaller aerosols, but that some larger particles normally classified as droplets can remain airborne

and be transported inside building such as LTCFs.[31] In addition, contamination of no-touch surfaces likely involves larger particles or droplets given their ability to settle. In that context, improper ventilation could contribute to viral accumulation in these environments.[31]

Early in the pandemic, concerns about transmission risks in Canadian healthcare settings were focused on acute care units. The massive outbreaks among long term care facilities and workers as the pandemic progressed have emphasized the very high risk of transmission in these crowded congregate environments. Viral contamination of no-touch surfaces supports that the aerosol route of transmission may be of importance in LTCFs and, despite being acknowledged at low risk for airborne viral exposure, that they should be reconsidered at a similar risk to hospitals and other healthcare environments, especially in the context of poor ventilation. Further work is needed to identify the modes of transmission of SARS-CoV-2 in LCTFs and to determine what practices are most important in preventing the propagation of the illness in residents and staff. In depth analyses and randomized trials looking into the benefits and applicability of the different personal and respiratory protections available for LTCFs healthcare workers seem necessary.

CONCLUSION

SARS-CoV-2 could not be detected in air samples from residents' room in 7 different LTCFs from 8 to 30 days after symptoms onset. However, viral genomes were recovered from settled dust on no-touch surfaces, suggesting viral dissemination in the environment through air had happened previous to sampling. This could be an indication of the importance of timing between the patients' stage of infection and air sampling deployment. The collaboration of LTCFs is deemed crucial in future work in order to access these establishments in a timely manner and to allow the collection of environmental data in the potential peak of exposition risks.

A moderate relation between positivity of surfaces swabs and the absence of mechanical ventilation was also observed and is in line with propositions from recent work about the control of airborne concentration of SARS-CoV-2 in indoor/closed spaces. Studies designed to assess the importance of ventilation in preventing accumulation of viral particles in the environment should be conducted to clearly demonstrate the importance of adequate indoor air management that could be beneficial to both residents and workers of LCTFs and other environments, such as schools.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2021.02.004.

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