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Duration of SARS-CoV-2 positive in quarantine room environments: A perspective analysis

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ABSTRACT

Objective: To determine the duration of SARS-CoV-2 persistence in quarantine hotel environments.
Methods: 39 Patients confirmed by RT-PCR were included. We collected clinical features, laboratory test results, smear sample information, and quarantine room information. Genome sequencing and phylogenetic analysis were conducted. We analyzed the factors associated with environmental contamination.
Result: Among 39 COVID-19 cases, 10 were asymptomatic and 37 were imported from aboard. We collected 271 swab samples from environmental surfaces related to observational patients. Eighteen swab samples from seven patients were positive. The highest contamination rates occurred on cups (100%), followed by hand sink (12.82%), toilet seat and flush (7.89%), telephone (5.56%), bedside table (5.56%), and floor drain (5.41%). The results showed that environmental surface contamination was associated with the clinical cycle threshold values for patients ($P = 0.01$) and the sampling interval time after the cases left their rooms ($P = 0.03$). The duration of environmental surface contamination was associated with the wet status of the sampling site ($P = 0.01$).
Conclusion: Our findings showed that environmental contamination might be attributed to the viral load in the respiratory tracts of patients and the sampling interval time after the cases left their rooms. Moist surfaces were more vulnerable to remaining SARS-CoV-2 RNA-positive. Our study highlights the importance of implementing strict chemical disinfection strategies in quarantine rooms.

Introduction

Coronavirus disease 2019 (COVID-19) has spread worldwide and over 34.8 million cases were confirmed in nearly 10 months during 2020 (WHO, 2020). The main transmission routes are through respiratory droplets and close contact (Chu et al., 2020). Exposure to virus-contaminated objects can also cause infection (Karia et al., 2020). There is a possibility of aerosol transmission in relatively closed environments exposed to high concentrations of aerosols for a long time (Jayaweera et al., 2020). A recent review also showed that there is evidence for the airborne transmission of coronavirus infection (Niazi et al., 2020). Coronavirus can be separated in feces or urine, so contact transmission or aerosol transmission may occur due to environmental contamination (Kang et al., 2020).

The possibility that sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can contaminate environments has attracted much attention worldwide. Colaneri et al. found positive samples of low-level SARS-CoV-2 RNA in continuous positive airway

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pressure helmets used by patients in an emergency acute health care unit in Italy (Colaneri et al., 2020). Razzini et al. detected SARS-CoV-2 RNA positive swab samples from hand sanitizer dispensers, medical equipment, medical equipment touch screens, and shelves for medical equipment in the COVID-19 ward of a hospital in Italy (Razzini et al., 2020). A study in the epicenter of Wuhan reported severe contamination in an intensive care unit (ICU) and an obstetric isolation ward specialized for pregnant women (Ye et al., 2020). Faridi et al. tested the air in the rooms of confirmed COVID-19 patients to determine whether SARS-CoV-2 RNA could be detected in aerosols but all of the samples were negative (Faridi et al., 2020). Lei et al. collected air and surface samples from a hospital that cared for severe COVID-19 cases in China, and air samples from the ICU and isolation ward contained SARS-CoV-2 RNA (Lei et al., 2020).

Several studies have investigated contamination of the environment before COVID-19 patients were admitted to hospital. In particular, SARS-CoV-2 RNA was detected on the pillow covers, sheets, and duvet covers in a quarantine hotel where people stayed after entering China from abroad (Jiang et al., 2020). SARS-CoV-2 contamination has also been detected in the daily living environments of patients, including surfaces in toilets, anterooms, kitchens, bedrooms, and door handles in their homes and cars (Luo et al., 2020). In addition, both of the aforementioned studies found that people infected with SARS-CoV-2 shed virus and contaminated the environment while they were asymptomatic. SARS-CoV-2 could spread through the drains in high-rise building in the form of fecal aerosols to cause a COVID-19 outbreak (Kang et al., 2020). In addition, a recent study showed that the virus remained viable in aerosols and it was more stable on plastic and stainless steel surfaces under experimental conditions (van Doremalen et al., 2020).

However, to the best of our knowledge, no previous studies have determined the duration of SARS-CoV-2 contamination in quarantine hotel environments. Thus, in the present study, we analyzed the duration of SARS-CoV-2 contamination in a quarantine environment.

Methods

Study design

This study was conducted in Guangzhou, China from March 8 to June 13, 2020. Patients confirmed by fluorescent real-time PCR as SARS-CoV-2 positive during quarantine after their entry into China from abroad were included. We collected clinical features, laboratory test results, sample collection information, and quarantine room information.

Sampling

Surface smear samples were collected from each quarantine room and placed in COPAN UTM-RT viruses transport medium, including samples from door handles, electric switches, cups, telephones, TV remote controls, bedside tables, bathroom door rails, hand sinks, toilet seats and flushes, and floor drains in bathrooms, as shown in Figure 1. If an environmental sample tested positive, personnel sampled the positive area again within 24 h. Sampling was repeated until the area tested negative three consecutive times. The surface of each sample area was swabbed with a separate swab and sent to the laboratory in virus preservation solution to detect SARS-CoV-2 virus RNA by RT-PCR. During the sampling and observation process, in order to form a relatively closed space, the doors and windows of the rooms were closed and the air conditioning was turned off. The indoor temperature and indoor relative humidity were recorded. The differences in the indoor temperature and indoor relative humidity between the second sampling visit and the previous sampling visit were calculated.

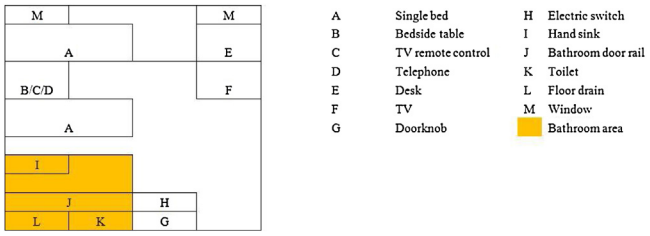

Figure 1. The quarantine room for people entering from abroad, with one room for each person.

Laboratory methods

(I) Novel coronavirus nucleic acid detection by quantitative real-time PCR

RNA was extracted with a Viral Nucleic Acid Isolation Kit (Magnetic Beads). Quantitative real-time PCR was conducted using a Novel Coronavirus 2019 Nucleic Acid Test Kit (bioPerfectus Technologies, Taizhou, China) and an Applied Biosystems ViiA7 instrument (Applied Biosystems, Hong Kong, China), according to the manufacturers' instructions. A TaqMan-probe-based kit was designed to detect ORF1ab and the N gene of SARS-CoV-2 in a single reaction. Thermal cycling was performed at 50 °C for 10 min for reverse transcription, followed by 97 °C for 1 min, and then 45 cycles at 97 °C for 5 s and 58 °C for 30 s. The cycle threshold (Ct) value determined from the amplification curve was defined as positive when it was less than 40 and negative when it exceeded 40. Each test routinely included positive and negative controls.

(II) Gene sequencing

Genome sequencing was performed for samples using the multiplex PCR approach with 98 pair primers each of 400 bp according to the ARTIC protocol (GitHub, 2020). The PCR products were pooled for library construction according to the manual for the ligation sequencing kit (SQK-LSK109) and sequenced with a MinION sequencer using the R9.4.1 flow cell.

Raw reads were then remapped to the assembly using the BWA program (v0.7.12) and alignments were processed with SAMtools (v1.3) to determine the consensus sequence (Li and Durbin, 2009; Li et al., 2009).

A maximum-likelihood phylogenetic tree was constructed using RAxML (v8.2.4) with a general time reversible model and a gamma distribution based on 1000 bootstraps (Stamatakis, 2014). The phylogenetic tree was visualized using iTOL (v4) (Letunic and Bork, 2019).

Statistical analysis

Descriptive analysis was conducted to calculate the proportions of each categorical variable to evaluate the contamination. Continuous variables were calculated for the quantity and median, and the variables were classified to determine the frequencies. We analyzed the factors associated with environmental contamination using Wilcoxon's rank-sum test for continuous variables, and the χ^2 test or Fisher's exact test for comparing categorical variables (Chia et al., 2020). *P*-values less than 0.05 were considered to indicate statistically significant differences. All analyses were conducted using SPSS 13.0.

Ethics statement

Clinical data on cases and the collection of health conditions at the isolation point were part of the investigation and control of the outbreak and informed consent was waived. Data were collected

by the Guangzhou Center for Disease Control and Prevention using a standardized anonymous structured questionnaire without patient identifiers.

Results

Indoor temperature and relative humidity

During the sampling period, the median indoor temperature was 26 °C (interquartile range (IQR): 21 °C to 32 °C) and the median indoor relative humidity was 60% (IQR: 45–71%). The median indoor temperature difference was 0 °C (IQR: –1 °C to 1 °C) and the median indoor relative humidity difference was 0% (IQR: –5% to 6%).

Characteristics of 39 COVID-19 cases

Table 1 shows the characteristics of the 39 COVID-19 cases investigated in our study, where 27 cases came from Asia and nine cases were tested by a third-party testing institution, and their Ct values were not available. Thus, the sample size for the Ct value statistics was defined as 30 cases, with a median value of 30.5 (IQR: 25.5–35).

Distribution of environmental contamination associated with 39 COVID-19 cases

In total, 271 swab samples were collected from environmental surfaces related to the observational patients mentioned above (Table 2). Eighteen (6.64%) swab samples from seven (17.95%) patients were SARS-CoV-2 RNA positive. Overall, cups were most likely to be contaminated (100%, 2/2), followed by hand sinks (12.82%, 5/39) and toilet seats and flushes (7.89%, 3/38). Different degrees of contamination were detected on telephones (5.56%, 1/18), bedside tables (5.56%, 2/36), floor drains (5.41%, 2/37), TV remote controls (4.35%, 1/23), bathroom door rails (3.23%, 1/31), and door handles (2.56%, 1/39).

Statistically significant differences were found in the clinical Ct value (P = 0.01) and the sampling interval time after the cases left the room (P = 0.03) for the positive environment and negative environment. However, there were no significant differences in gender, pulmonary inflammation, and onset clinical symptoms (Table 3).

Table 1
Description of 39 observational COVID-19 cases characteristic.

Item	N	%
Gender		
Males	21	53.85
Females	18	46.15
Classification		
Confirmed cases	29	74.36
Asymptomatic cases	10	25.64
Clinical severity		
Asymptomatic	10	25.64
Mild	9	23.08
Moderate	20	51.28
Clinical symptoms		
Pulmonary imaging inflammation	23	58.97
Fever	5	12.82
Cough(including dry cough)	14	35.90
Throat discomfort (including pharyngoxerosis, pharyngalgia, pharyngeal itching)	11	28.21
Nasal discomfort (including blocked or runny nose)	5	12.82
Imported from[a]		
Bangladesh	16	41.03
Philippines	3	7.69
Cambodia	2	5.13
Pakistan	2	5.13
Thailand	2	5.13
India	1	2.56
Malaysia	1	2.56
Britain	3	7.69
France	2	5.13
Spain	1	2.56
America	3	7.69
Canada	1	2.56

[a] Other two cases were imported associated cases.

Continuous contamination of environmental surfaces with SARS-CoV-2 RNA

The median Ct value for SARS-CoV-2 RNA positive surface swab samples was 35 (IQR: 34–36.5). The median number of days with continuous SARS-CoV-2 RNA contamination on environmental surfaces was 5 (IQR: 1–15.5). The longest number of days when virus RNA was continuously detected was determined for the hand sink samples (median: 19, IQR: 12.5–26.25) (Figure 2).

Comparisons of the sampling locations and days with persistent contamination showed that the continuous contamination of environmental surfaces with SARS-CoV-2 RNA was associated with the wet status of the sampling site (P = 0.01). However, there were no significant differences in the Ct values for the surface swabs (P = 0.356).

Gene sequencing

Among the seven respiratory samples associated with continuous environmental contamination, four samples were suitable for gene sequencing after establishing our database and quality control. Figure 3 shows the phylogenetic tree established for the genetic sequences. Sample A (42352) belonged to L clade, pedigree B, and its sequence was found closely related to a coronavirus genome collected in mainland and Taiwan China. The longest contamination period with this virus was detected on a cup, with 5 days. Sample B (42961) and sample C (43432) belonged to O clade, pedigree B.6, and the sequences of these two samples were closely related to a coronavirus genome collected in Kwan Island, Taiwan China, and India. The two samples with the longest contamination by these viruses were collected from wash basins, with periods of 28 and 21 days. Sample D (45796) belonged to S clade, pedigree A6, and its sequence was closely related to a genome collected in Thailand. The longest contamination with this virus occurred on a wash basin, with 17 days.

Discussion

COVID-19 is still a global pandemic and the resumption of flights means that the quarantine and isolation of imported cases are important components of epidemic prevention and control. Guangzhou municipal government implemented a preventive measure from March 8, 2020, requiring centralized isolation for people who enter from abroad (Zhang et al., 2020). After the isolation period is complete or a person is hospitalized, the quarantine room must be sampled for pathogenic viruses before and after terminal disinfection by professional technicians. The degree of contamination and terminal disinfection in the quarantine rooms used for confirmed or asymptomatic cases are important for the disinfection personnel.

In the present study, we found that the environmental surfaces in quarantine rooms used for people who entered from abroad were frequently contaminated with SARS-CoV-2 virus. The contaminated sites included hand sinks, bathroom door rails, toilet seats and flushes, floor drains, cups, bedside tables,

Table 2
Description of 271 swab samples of environmental surfaces characteristic.

Item	Sampling specimens	Percentage in total (%)	Positive sampling specimens	Percentage positive (%)
Sampling locations				
Cup	2	0.74	2	100.00
Hand sink	39	14.39	5	12.82
Toilet seat and flush	38	14.02	3	7.89
Telephone	18	6.64	1	5.56
Bedside table	36	13.28	2	5.56
Floor drain	37	13.65	2	5.41
TV remote control	23	8.49	1	4.35
Bathroom door rail	31	11.44	1	3.23
Doorknob	39	14.39	1	2.56
Electric switch	8	2.95	0	0.00
Materials				
Wood	31	11.44	2	6.45
Plastic	55	20.30	2	3.64
Ceramics	78	28.78	10	12.82
Metal	107	39.48	4	3.74

Table 3
Factors associated with environmental contamination in COVID-19 cases.

Characteristics of patients	Environmental contamination of rooms				P value
	Positive(n = 7)		Negative(n = 32)		
CT examination showed pneumonia (%)	5	(12.82)	18	(46.15)	0.68
Males (%)	6	(15.38)	15	(38.46)	0.10
Symptomatic (%)	6	(15.38)	23	(58.97)	0.65
Time from isolation to hospitalization (hour), median (IQR)	26	(25–62)	34.5	(25–36)	0.46
Time of sampling interval after case left room (hour), median (IQR)	10	(2–13)	13	(13–15)	0.03
Clinical sample cycle threshold value, median (IQR)	24	(23–28)	33	(28–35)	0.01

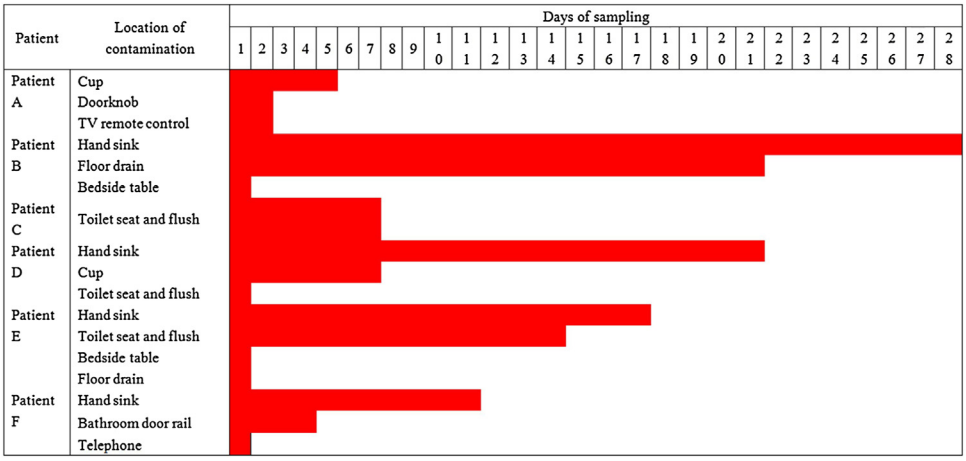

Figure 2. Days of continuous SARS-CoV-2 RNA contamination in environmental surface. Note:1 (1/18) sample of washbasin was thoroughly cleaned and disinfected after testing positive due to on-site health prevention and control requirements, so it was not put into the observation cohort for the duration study of contamination.

telephones, TV remote controls, and door handles. Previous studies also provided evidence (Jiang et al., 2020; Luo et al., 2020) that the environments where patients resided before hospitalization were a risk for transmission through contaminated surfaces, especially asymptomatic cases. It has been shown that SARS-CoV-2 virus is contagious during the incubation period (Wiersinga et al., 2020) and highly contagious for 5 days at the beginning of the COVID-19 illness (Wölfel et al., 2020), which may support our findings. The most common symptoms of infection with SARS-CoV-2 virus are fever, fatigue, and a dry cough, and some patients will develop less common symptoms, such as sputum production, nausea, and vomiting (Hu et al., 2020). These symptoms can readily lead to environmental contamination in quarantine rooms. The most frequently contaminated site was cups (100%), which is understandable because saliva has a high viral load. In addition, frequent contamination was found on the surfaces of hand sinks (12.82%) and the duration was relatively long, with at least 11–28 days according to our results. The virus might have readily contaminated sinks when patients gargled or spat into them. It should be noted that the relative humidity and absolute humidity are associated with the viability of airborne respiratory viruses (Niazi et al., 2020). The sinks were often humid and the temperature was suitable, thereby facilitating the possible spread of the virus. However, few studies have investigated this source of the virus and this risk is generally ignored, probably because running the tap water frequently is assumed to reduce the risk. Moreover, it is

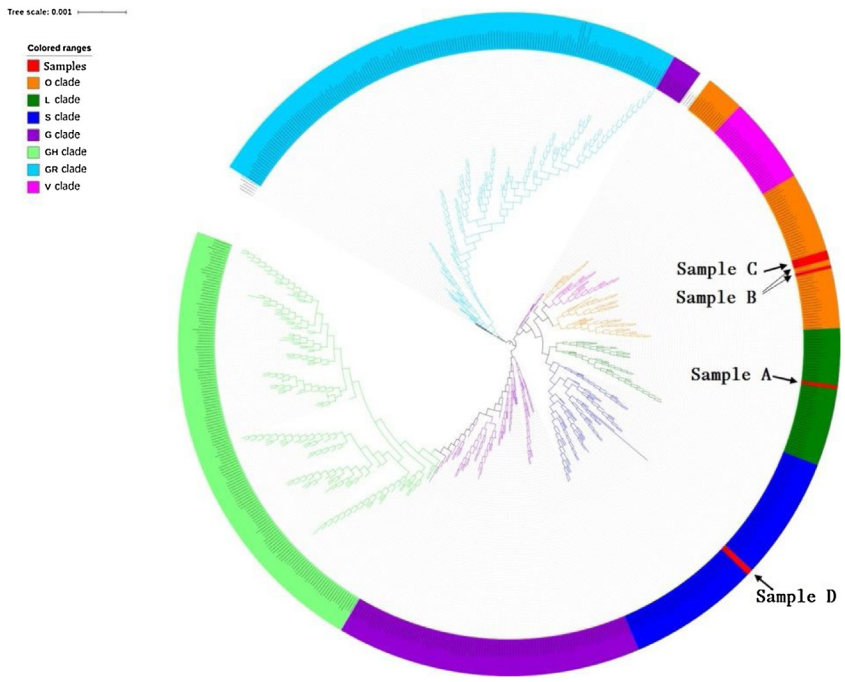

Figure 3. Phylogenetic trees of genetic sequences. The red color refers to amplicon fragments of RNA-dependent RNA polymerase of sample A (42357), B (42961), C (43432), and D (45796).

interesting that although no cases of gastrointestinal symptoms were reported in our study, viral nucleic acids were detected on the surfaces of the toilets and flushes (7.89%). In general, SARS-CoV-2 virus tended to contaminate surfaces that are often ignored, thereby potentially risking infection by daily contact. The common contaminated sites and surfaces mentioned above should be subjected to disinfection with high concentration chlorine-releasing agents, e.g., at concentrations over 1000 mg/L.

Thus, our findings demonstrate that the quarantine rooms used for preventing the transmission of COVID-19 should be cleaned and disinfected thoroughly. Studies have showed that exposure to a high concentration of SARS-CoV-2 virus aerosols in a relatively closed environment can potentially allow aerosol transmission (Fisher et al., 2020; Lu et al., 2020). Thus, disinfecting the air with peroxyacetic acid, hydrogen peroxide, or other chemicals should be considered. Another important issue is that personnel, including routine cleaners, sterilizers, and other workers who maintain quarantine room operations, must strictly follow personal protection procedures, including wearing gloves, masks, glasses, and protective clothing. Similar suggestions were made in a study based on indoor air measurements in Iran (Faridi et al., 2020). Furthermore, the terminal disinfection of quarantine rooms should be conducted strictly in accordance with the corresponding work standards, and the effectiveness of the disinfection process should be evaluated regularly. Given these considerations, we recommend the centralized isolation of close contacts or people with the requirement for quarantine.

In addition, we assessed the relationship between the environmental contamination caused by patients and the viral load in the respiratory tracts of patients, as also determined in a previous study (Chia et al., 2020). Moreover, we provided evidence that surface contamination was not associated with the time from isolation to hospitalization, but instead it was related to the sampling interval time after people left the room, which may be attributed to the stability of the SARS-CoV-2 virus in the environment (van Doremalen et al., 2020).

A previous study showed that the SARS-CoV-2 virus can remain viable in aerosols for 3 h as well as being detected for up to 72 h on plastic and stainless steel surfaces (van Doremalen et al., 2020). An experimental study also showed that the virus could remain highly stable in a favorable environment (Chin et al., 2020). In addition, an investigation of airborne common cold viruses showed that some airborne viruses, such as influenza, can survive and remain stable in the relative humidity range that is suitable for humans (Niazi et al., 2021). However, we found that SARS-CoV-2 RNA could be continuously detected on a wet ceramic material for several days and even up to 28 days. The persistence of the virus was related to the wetness and texture of the contaminated surface. To the best of our knowledge, this is the first study to demonstrate the sustained detection of SARS-CoV-2 RNA on environmental surfaces under actual working conditions. Yao et al. elucidated the natural structure and assembly mechanism of the novel coronavirus complex, which are important for the long-term survival of the SARS-CoV-2 virus in the external environment (Yao et al., 2020). The viability of the desiccated virus is affected by a variety of environmental conditions and parameters, such as the temperature, humidity, pH, and surface type (Firquet et al., 2015). In particular, the humidity as the amount of water in the ambient air is a strong determinant of the spread of other viruses in the environment (Wolkoff, 2018). An experimental study showed that human rhinoviruses have different survival rates in aerosols under different relative humidity levels (Niazi et al., 2021). Most viruses spread by the fecal–oral route are highly resistant to exposure to water, which may support our finding that SARS-CoV-2 RNA could persist for a long time on wet surfaces, such as hand sinks and floor drains.

Several studies detected SARS-CoV-2 RNA in feces and sewage (Amirian, 2020; Chen et al., 2020; Xing et al., 2020), and others found infectious coronavirus particles in feces (Pan et al., 2020; Wang et al., 2020). Thus, exposure to untreated sewage or waste water might be a potential infection risk for people. Moreover, the sewage from toilets, sinks, and floor drains is discharged into municipal sewage treatment systems, and previous studies have detected SARS-CoV-2 RNA in untreated or treated wastewater (Ahmed et al., 2020; Haramoto et al., 2020; Randazzo et al., 2020; Wu et al., 2020). Our findings indicate that the long-term residual

presence of virus in wastewater may represent a hidden danger if disinfection is not sufficiently thorough. Therefore, we suggest the implementation of devices to ensure the continuous disinfection of sewage discharge equipment in quarantine rooms, including hand sinks, toilets, and floor drains.

Our study had some limitations. First, our study was conducted in the context of epidemic prevention and control, so the sample size was limited. Second, repeated sampling at the same location to determine the presence of continuous contamination might have reduced the viral concentration, thereby leading to possible differences between the observed results and the actual results. Finally, similar to most other SARS-CoV-2 environmental contamination studies, the SARS-CoV-2 RNA positive samples collected from environmental surfaces could not be cultivated to isolate the viruses, thereby weakening the evidence for transmission via contaminated environmental surfaces.

Conclusion

In this study, we evaluated the locations of environmental contamination with SARS-CoV-2 in quarantine rooms before hospitalization and the continuous duration of contamination associated with COVID-19 cases. We found that environmental contamination could be attributed to the viral load in the respiratory tracts of patients and the sampling interval after they left their rooms. Moist surfaces were vulnerable to remaining SARS-CoV-2 RNA positive. Our findings provide basic knowledge to support the implementation of a strict disinfection strategy for quarantine rooms and for improving the effective control and prevention of COVID-19.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript did not contain any individual person's data.

Competing interest

There are no competing interests to declare.

Availability of data and materials

Survey data for this study are available from Xiaowei Ma at 103073279@qq.com.

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CRediT authorship contribution statement

Jie Liu: Methodology, Investigation. **Jingwen Liu:** Writing - original draft, Visualization. **Zheng He:** Methodology, Investigation. **Zhicong Yang:** Conceptualization, Supervision. **Jun Yuan:** Conceptualization. **Haoying Wu:** Methodology. **Pingting Zhu:** Methodology. **Xuesong Fu:** Methodology. **Yunwan Lin:** Methodology. **Ying Zhang:** Methodology. **Zhengyang Zhao:** Methodology. **Shiyu He:** Data curation, Software. **Xiaowei Ma:** Writing - original draft, Writing - review & editing, Supervision.

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References

Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ 2020;728:138764, doi:http://dx.doi.org/10.1016/j.scitotenv.2020.138764.

Amirian ES. Potential fecal transmission of SARS-CoV-2: current evidence and implications for public health. Int J Infect Dis 2020;95:363–70, doi:http://dx.doi.org/10.1016/j.ijid.2020.04.057.

Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol 2020;92:833–40, doi:http://dx.doi.org/10.1002/jmv.25825.

Chia PY, Coleman KK, Tan YK, Ong SWX, Gum M, Lau SK, et al. Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients. Nat Commun 2020;11:2800, doi:http://dx.doi.org/10.1038/s41467-020-16670-2.

Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen HL, Chan MCW, et al. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 2020;1:e10, doi:http://dx.doi.org/10.1016/s2666-5247(20)30003-3.

Chu DK, Akl EA, Duda S, Solo K, Yaacoub S, Schünemann HJ. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. Lancet 2020;395:1973–87, doi:http://dx.doi.org/10.1016/s0140-6736(20)31142-9.

Colaneri M, Seminari E, Novati S, Asperges E, Biscarini S, Piralla A, et al. Severe acute respiratory syndrome coronavirus 2 RNA contamination of inanimate surfaces and virus viability in a health care emergency unit. Clin Microbiol Infect 2020;26:1094.e1–5, doi:http://dx.doi.org/10.1016/j.cmi.2020.05.009.

Faridi S, Niazi S, Sadeghi K, Naddafi K, Yavarian J, Shamsipour M, et al. A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran. Sci Total Environ 2020;725:138401, doi:http://dx.doi.org/10.1016/j.scitotenv.2020.138401.

Firquet S, Beaujard S, Lobert PE, Sané F, Caloone D, Izard D, et al. Survival of enveloped and non-enveloped viruses on inanimate surfaces. Microbes Environ 2015;30:140–4, doi:http://dx.doi.org/10.1264/jsme2.ME14145.

Fisher KA, Tenforde MW, Feldstein LR, Lindsell CJ, Shapiro NI, Files DC, et al. Community and close contact exposures associated with COVID-19 among symptomatic adults ≥18 years in 11 outpatient health care facilities – United States, July 2020. MMWR Morb Mortal Wkly Rep 2020;69:1258–64, doi:http://dx.doi.org/10.15585/mmwr.mm6936a5.

GitHub. artic-ncov2019. [cited 2020-10-31]; Available from:. 2020. https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019/V3.

Haramoto E, Malla B, Thakali O, Kitajima M. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci Total Environ 2020;737:140405, doi:http://dx.doi.org/10.1016/j.scitotenv.2020.140405.

Hu B, Guo H, Zhou P, Shi Z-L. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 2020;, doi:http://dx.doi.org/10.1038/s41579-020-00459-7.

Jayaweera M, Perera H, Gunawardana B, Manatunge J. Transmission of COVID-19 virus by droplets and aerosols: a critical review on the unresolved dichotomy. Environ Res 2020;188:109819, doi:http://dx.doi.org/10.1016/j.envres.2020.109819.

Jiang FC, Jiang XL, Wang ZG, Meng ZH, Shao SF, Anderson BD, et al. Detection of severe acute respiratory syndrome coronavirus 2 RNA on surfaces in quarantine rooms. Emerg Infect Dis 2020;26:2162–4, doi:http://dx.doi.org/10.3201/eid2609.201435.

Kang M, Wei J, Yuan J, Guo J, Zhang Y, Hang J, et al. Probable evidence of fecal aerosol transmission of SARS-CoV-2 in a high-rise building. Ann Intern Med 2020;173(12):974–80, doi:http://dx.doi.org/10.7326/m20-0928.

Karia R, Gupta I, Khandait H, Yadav A, Yadav A. COVID-19 and its modes of transmission. SN Compr Clin Med 2020;1–4, doi:http://dx.doi.org/10.1007/s42399-020-00498-4.

Lei H, Ye F, Liu X, Huang Z, Ling S, Jiang Z, et al. SARS-CoV-2 environmental contamination associated with persistently infected COVID-19 patients. Influenza Other Respir Viruses 2020;14(6):688–99, doi:http://dx.doi.org/10.1111/irv.12783.

Letunic I, Bork P. Interactive tree of life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 2019;47:W256–w259, doi:http://dx.doi.org/10.1093/nar/gkz239.

Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754–60, doi:http://dx.doi.org/10.1093/bioinformatics/btp324.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics 2009;25:2078–9, doi:http://dx.doi.org/10.1093/bioinformatics/btp352.

Lu J, Gu J, Li K, Xu C, Su W, Lai Z, et al. COVID-19 outbreak associated with air conditioning in restaurant, Guangzhou, China, 2020. Emerg Infect Dis 2020;26:1628–31, doi:http://dx.doi.org/10.3201/eid2607.200764.

Luo L, Liu D, Zhang H, Li Z, Zhen R, Zhang X, et al. Air and surface contamination in non-health care settings among 641 environmental specimens of 39 COVID-19 cases. bioRxiv 2020;14(10):e0008570, doi:http://dx.doi.org/10.1101/2020.07.09.195008.

Niazi S, Groth R, Cravigan L, He C, Tang JW, Spann K, et al. Susceptibility of an airborne common cold virus to relative humidity. Environ Sci Technol 2021;55:499–508, doi:http://dx.doi.org/10.1021/acs.est.0c06197.

Niazi S, Groth R, Spann K, Johnson GR. The role of respiratory droplet physicochemistry in limiting and promoting the airborne transmission of human coronaviruses: a critical review. Environ Pollut 2020;115767, doi:http://dx.doi.org/10.1016/j.envpol.2020.115767.

Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020;20:411–2, doi:http://dx.doi.org/10.1016/s1473-3099(20)30113-4.

Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res 2020;181:115942, doi:http://dx.doi.org/10.1016/j.watres.2020.115942.

Razzini K, Castrica M, Menchetti L, Maggi L, Negroni L, Orfeo NV, et al. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. Sci Total Environ 2020;742:140540, doi:http://dx.doi.org/10.1016/j.scitotenv.2020.140540.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 2014;30:1312–3, doi:http://dx.doi.org/10.1093/bioinformatics/btu033.

van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020;382:1564–7, doi:http://dx.doi.org/10.1056/NEJMc2004973.

Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843–4, doi:http://dx.doi.org/10.1001/jama.2020.3786.

WHO. Weekly Epidemiological Update. [cited 2020-10-08]; Available from:. 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20201005-weekly-epi-update-8.pdf.

Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782–93, doi:http://dx.doi.org/10.1001/jama.2020.12839.

Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465–9, doi:http://dx.doi.org/10.1038/s41586-020-2196-x.

Wolkoff P. Indoor air humidity, air quality, and health – an overview. Int J Hyg Environ Health 2018;221:376–90, doi:http://dx.doi.org/10.1016/j.ijheh.2018.01.015.

Wu F, Zhang J, Xiao A, Gu X, Lee WL, Armas F, et al. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. mSystems 2020;5:, doi:http://dx.doi.org/10.1128/mSystems.00614-20.

Xing YH, Ni W, Wu Q, Li WJ, Li GJ, Wang WD, et al. Prolonged viral shedding in feces of pediatric patients with coronavirus disease 2019. J Microbiol Immunol Infect 2020;53:473–80, doi:http://dx.doi.org/10.1016/j.jmii.2020.03.021.

Yao H, Song Y, Chen Y, Wu N, Xu J, Sun C, et al. Molecular architecture of the SARS-CoV-2 virus. Cell 2020;183(3):730–8, doi:http://dx.doi.org/10.1016/j.cell.2020.09.018.

Ye G, Lin H, Chen S, Wang S, Zeng Z, Wang W, et al. Environmental contamination of SARS-CoV-2 in healthcare premises. J Infect 2020;81:e1–5, doi:http://dx.doi.org/10.1016/j.jinf.2020.04.034.

Zhang ZB, Li L, Qin PZ, Li K, Huang Y, Luo L, et al. Countries of origin of imported COVID-19 cases into China and measures to prevent onward transmission. J Travel Med 2020;27(8):taaa139, doi:http://dx.doi.org/10.1093/jtm/taaa139.