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BRIEF REPORT – OPEN ACCESS

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Monitoring of SARS-CoV-2 RNA in Public Areas: An Investigation of Environmental Surface Contamination

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ABSTRACT

Although droplets and aerosol are considered the main transmission routes of SARS-CoV-2, indirect contact has been indicated to play a critical role in transmission. The aim of this study is to evaluate the presence of SARS-CoV-2 RNA on different environmental surfaces in public areas in Cyprus. Using RT-qPCR, samples from 50 swab specimens collected from high-touch surfaces were analyzed for viral RNA. Six surfaces (12.0%) in all were positive for SARS-CoV-2. Among the examined surfaces within supermarkets, SARS-CoV-2 was detected in 22.2% (n=4/18) of the sampling points, with shopping trolley handles and POS keyboards being the most frequently contaminated items. In the hospital setting, two (n=2/5, 40%) samples were positive for SARS-CoV-2. Our results indicate that, at the current stage of the pandemic, viral contamination of public spaces exists in the community. Lifting protective measures may have contributed to fomite transmission in public spaces.

Keywords: SARS-CoV-2, RT-qPCR, environmental contamination, Cyprus, public areas

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INTRODUCTION

Since its emergence in 2019, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that caused COVID-19 has spread rapidly, with over 600 million cases and more than 6.4 million fatalities reported as of September 2022 (1). The primary mode of transmission for COVID-19 is through direct contact with an infected individual via inhalation of droplets and aerosols carrying SARS-CoV-2. Indirect transmission occurs through contact with contaminated surfaces (2, 3). Although the primary focus is mostly on transmission via direct contact with an infected individual, recent findings indicate that indirect (fomite) transmission routes can also have a significantly impact on the spread of SARS-CoV-2.

Detection of SARS-CoV-2 in environmental surfaces has highlighted the importance of fomite transmission routes for COVID-19. Contamination rates and viral loads are particularly high in healthcare settings (4, 5). A study by Chia et al. (4) indicated that at least one SARS-CoV-2 contaminated surface was found in 56.7% of the rooms inhabited by COVID-19-positive patients in a hospital. Two independent studies performed in Brazil and Italy described the presence of SARS-CoV-2 on surfaces of non-healthcare-related public areas, including benches, door handles, handrails, and bar counters (5, 6).

SARS-CoV-2 was reported to persist on multiple surfaces for a significant time under certain conditions. The viability and stability of the virus depend on various conditions. Humidity and temperature play a crucial role in the viability of SARS-CoV-2; the survival time of the virus significantly increases when it is exposed to lower temperature and humidity (7). Another determining variable for viral viability is the material of the surface. Viable SARS-CoV-2 is detected on stainless-steel, and plastic surfaces up to 3 days after treatment with the virus (8), while viable SARS-CoV-2 was detected up to 21 days and 14 days post-treatment for plastic and stainless-steel surfaces, respectively (9).

Considering the circulation of the highly transmissible SARS-CoV-2 Omicron BA.2 subvariant in Cyprus during the study period, we aim to investigate the presence of SARS-CoV-2 RNA on local public spaces and assess whether surface contamination exists in the current state of the pandemic.

MATERIALS and METHODS

Ethical Approval

This study was approved by the Institutional Review Board at Near East University (Project No: YDU/2022/102-1546).

Environmental Sampling: Specimen Collection, Storage, and Transfer

This was a cross-sectional study. In this study, 50 samples were collected from 7 critical points in the community between February and March 2022. The collection locations included two supermarkets (in two different regions of the city), two pharmacies, a private hospital, multiple ATMs located throughout the city, a bank, and a faculty and student school bus. The samples were collected from different high-touch surfaces, including shopping trolley handles, door handles, door knobs, handrails, bus handles, elevator buttons, POS keyboards, ATM counters, cashier keyboards, shelves, and cabins. The surfaces were sampled using sterile swabs placed into tubes containing 3 mL of the viral transport medium (VTM) (Hibrigen, Türkiye). For each sampling, a moistened swab was used with sliding and rotating movements in at least two directions for effective sample collection. Sampling time was chosen to represent a high circulation of individuals, such as between 9 a.m. and 12 a.m. for supermarkets and bank ATMs and until 5 p.m. for hospital, pharmacy, and faculty environments. The swab samples were transferred to the laboratory at 4°C and immediately processed.

RT-qPCR Analysis of SARS-CoV-2 RNA

Tubes containing samples in VTM were vortexed for 20 s for lysis according to the manufacturer's instructions. RT-qPCR was performed from the lysates using COVID-19 RT-PCR Kit (Hibrigen, Türkiye). A 14 µl mastermix containing the primer-probe mix and 6 µl sample was used for amplification using Q96TM Plus Real-time PCR Machine. Detection of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes was performed. An internal control (RPII gene) amplification was used in the RT-qPCR to ensure sample collection quality and PCR analysis. Positive control and no template (negative) control were included in each PCR run. Each reaction was performed in duplicate. Cycling conditions were 55°C for 15 min; 95°C for 1 min; 94°C for 5 s; and 60°C for 20 s (45 amplification cycles). Samples with a cycle threshold (Ct) value <40 were reported positive for SARS-CoV-2.

RESULTS

A total of 50 environmental specimens collected from multiple sampling sites across Nicosia were investigated for SARS-CoV-2 contamination. Results obtained in the study from various sampling sites and surface types are given in Table 1. Among the collected samples, 6 (12%) of the environmental specimens were positive for SARS-CoV-2 RNA. Importantly, half of the positive samples (n=3) were taken from the supermarket trolley handles and were present in both supermarkets included in the study. One of the positive samples was from a supermarket POS machine. Another sampling location detected to be positive for SARS-CoV-2 was the hospital. Both specimens obtained from the hospital counter and door knob were positive for viral contamination. The RT-qPCR amplification plots of the positive specimens are given in Figure 1.

DISCUSSION

Many studies centered around healthcare facilities have reported varying levels of environmental SARS-CoV-2 contamination (4, 10). Although the current study's primary aim was not to assess healthcare settings, our findings indicated surface contamination in the

Table 1. SARS-CoV-2 results according to sampling source and location

Sample no	Sample no	Result	Ct value
1.	Supermarket trolley	Negative	–
2.	ATM	Negative	–
3.	Supermarket POS machine	Negative	–
4.	Elevator button	Negative	–
5.	Pharmacy counter	Negative	–
6.	Supermarket trolley B	Negative	–
7.	ATM	Negative	–
8.	ATM	Negative	–
9.	ATM	Negative	–
10.	Supermarket POS machine	Negative	–
11.	Supermarket trolley C	**Positive**	26.61
12.	Supermarket door	Negative	–
13.	Bus door button	Negative	–
14.	Faculty door	Negative	–
15.	Bank door knob	Negative	–
16.	Bank door knob	Negative	–
17.	Pharmacy antigen test cabin	Negative	–
18.	Supermarket trolley	Negative	–
19.	ATM	Negative	–
20.	Supermarket trolley	Negative	–
21.	Supermarket food counter	Negative	–
22.	Supermarket shelf	Negative	–
23.	Supermarket till	Negative	–
24.	Supermarket POS machine	**Positive**	32.89
25.	Supermarket trolley	Negative	–
26.	Supermarket food counter	Negative	–
27.	ATM	Negative	–
28.	ATM	Negative	–
29.	Supermarket fridge door handle	Negative	–
30.	Supermarket trolley	**Positive**	30.97
31.	Supermarket trolley	**Positive**	33.74
32.	Pharmacy POS machine	Negative	–
33.	ATM	Negative	–
34.	Bus handle	Negative	–
35.	Supermarket POS machine	Negative	–
36.	Pharmacy door knob	Negative	–
37.	Hospital door knob	**Positive**	33.96
38.	Faculty door knob	Negative	–
39.	Hospital door knob	Negative	–
40.	Hospital door knob	Negative	–
41.	Supermarket trolley	Negative	–
42.	Bus handle	Negative	–
43.	Bus handle	Negative	–
44.	Faculty door knob	Negative	–
45.	Hospital counter	**Positive**	35.6
46.	Hospital counter	Negative	–
47.	Faculty elevator	Negative	–
48.	Supermarket entrance doorknob	Negative	–
49.	Bus handle	Negative	–
50.	Bus handle	Negative	–

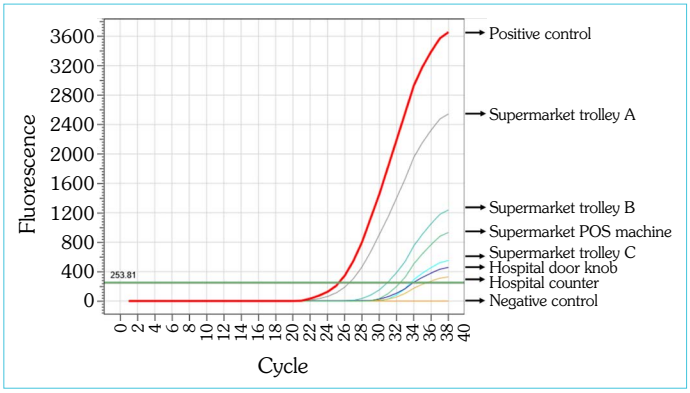


Figure 1. RT-qPCR amplification plots of SARS-CoV-2 positive specimens

hospital investigated. Out of the five samples obtained from surfaces within the hospital, two (n=2/5, 40%) specimens were positive for SARS-CoV-2. Among these, one of the positive samples was collected from a hospital counter, and the second one was collected from a doorknob with Ct values of 35.6 and 33.96, respectively. Environmental contamination of healthcare facilities poses a significant risk for healthcare workers and individuals belonging to risk groups who frequently visit hospitals. It should also be noted that door knobs and counters are both high-touch surfaces and might play an important role in the transmission of COVID-19. Considering these findings, it can be stated that the routine decontamination of surfaces still plays a key role as a preventive measure against COVID-19.

Our results showed considerable surface contamination in supermarkets. Among the eighteen surfaces within supermarkets examined, SARS-CoV-2 was found in 22.2% (n=4/18) of them, representing 66.6% (n=4/6) of the positive samples. A study performed on surface contamination in supermarkets during a lockdown in Italy reported a positivity rate of 4.3%, which is significantly lower than our findings (11). This might indicate a rise in viral surface contamination as the protective measures and restrictions to stop the spread of SARS-CoV-2 ease. There are no studies regarding SARS-CoV-2 environmental contamination during the March 2020 lockdown period in Cyprus. However, studies performed in various parts of the world during lockdowns or with relatively more strict measures in place also demonstrated lower positivity rates (<10%) than our findings (5, 6). As of March 2022, many of the protective measures, such as the compulsory use of masks in public spaces or screening for PCR/antigen test results or vaccination cards during entry, have been lifted in Cyprus, and the same shift can be observed in supermarket policies on routine disinfection of surfaces. Following these changes, an upsurge in the number of new cases has been observed. In addition, the decontamination and disinfection protocols in supermarkets, including hand sanitizers at the entrance and routine disinfection on trolley handles after each customer use, have been recently eased. Lifting these protective measures in supermarkets in Cyprus may have facilitated the fomite transmission in these public areas.

SARS-CoV-2 Omicron BA.2 subvariant was the predominant variant of concern circulating in Cyprus during the study period, February-March 2022, making up 70–85% of all positive cases [unpublished data]. The SARS-CoV-2 BA.2 subvariant's highly contagious nature could contribute to the environmental contamination observed in this study (12).

Our findings did not show insights into the infectivity or viability of SARS-CoV-2 detected on contaminated surfaces; however, prior studies have shown that SARS-CoV-2 can stay viable for prolonged periods, particularly on smooth surfaces such as plastic and stainless steel (8). Most of the positive samples obtained in the study were from plastic surfaces, on which SARS-CoV-2 can potentially stay viable for up to 4 days (22°C, 65% RH) (13). Further research would be required to establish the infectivity of the viral particles detected.

Our results revealed surface contamination in Cyprus public spaces, indicating that fomite transmission of SARS-CoV-2 remains a possible threat in the current pandemic state. While there are fewer restrictions on COVID-19 prevention worldwide, it is crucial to emphasize the importance of environmental and personal hygiene as protective measures against the disease.

Limitations

A major limitation of this study was the lack of viral viability testing. Additional limitations include the lack of humidity or temperature measurements at the sampling locations and the small sampling size. The results of the study might be enhanced by a subsequent investigation using more samples.

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