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Investigation of SARS-CoV-2 presence on environmental surfaces and waste in healthcare and non-healthcare facilities

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ABSTRACT
Objective: The objective of the paper is to investigate the presence of SARS-CoV-2 on inanimate surfaces in four healthcare facilities treating patients with COVID-19 and four quarantine regiments of provincial military commands.
Methods: From August to October 2020, a total of 468 one-off environmental samples consisting of inanimate surfaces, garbage, and wastewater were collected. The real-time RT-PCR assay targeting E and RdRp genes to detect SARS-CoV-2 and checklist and questionnaire of disinfection practices were employed. If detected by RT-PCR, then positive samples are subjected to cell culture to determine viability.
Results: The test results showed all samples (100%) to be negative with SARS-CoV-2 resulting in unperformed virus culture. As for recent disinfection practices, chlorine-based products dissolved at a concentration of 0.1% (1000 ppm) in the general context or 0.5% (5000 ppm) for blood and body fluid spills are routinely applied twice a day and at the discharge of patients or quarantined people.
Conclusions: The finding may illustrate the importance of disinfection practices in removing pathogens or significantly reducing SARS-CoV-2 contamination on environmental surfaces and waste.

1. Introduction

Since the COVID-19 pandemic is spreading worldwide, interventions to break the chain are crucial in for its transmission control. Several studies have succeeded in detecting SARS-CoV-2 survival on fomite surfaces, but the others did not (Barksdale et al., 2020; Kampf et al., 2020; Liu et al., 2021). The frequency of SARS-CoV-2 detection on surfaces in healthcare and non-healthcare settings and fomite transmission is not clearly known. Since the first case reported on 23 January 2020, Vietnam has experienced four waves of COVID-19 pandemic with more than 3000,000 cases and nearly 40,000 deaths (Vietnam Ministry of Health, 2020b). During the pandemic, all positive cases of COVID-19, symptomatic or asymptomatic, must be immediately referred to designated hospitals for its treatment and follow-up. In addition, all individuals who have contacted with positive cases or internationally entered Vietnam must be isolated in the quarantine centers for a minimum of 14 days (Nguyen et al., 2021; Vietnam Ministry of Health, 2020a). Concern has been raised about the presence and viability of SARS-CoV-2 in healthcare facilities and quarantine centers in Vietnam. The objective of this study was to determine if SARS-CoV-2 viral particles were present on inanimate surfaces in the healthcare facilities treating COVID-19 patients and quarantine centers in Vietnam.

2. Methods

2.1. Environmental surface sampling

One-off sampling was employed in four healthcare facilities involved in the management of COVID-19 patients and four regiments of provincial military commands worked as quarantine centers in four provinces in Vietnam. The number of current and cumulative COVID-19 positive patients and quarantined people were detailed in Fig. 1 and Fig. 2. Of note, current positive cases ranged from 1 to 20 and current quarantined people ranged from 26 to 109. Regarding COVID-19 positive patients, all of them underwent treatment at different stages of disease progression, that is, acute to resolving stages.

The environmental sampling sites and the technique were based on the World Health Organization (WHO) protocol on surface sampling for SARS-CoV-2 (WHO, 2020). Samples were collected at the entrance, corridors, elevators, anterooms (handrail, doorknob, operation panel),

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in the patient/isolation room (television remote control, light switch, oximeter, bed call button, floor and wall 1 m from patient’s bed, cup, chair, clothes, face mask, shoe sole, bedrail, doorknob, ventilation exit) and in the staff room (desk, chair, computer screen, key board, medical protective clothes, shoe sole, gloves, face mask, light switch, doorknob). The samples were collected in the patient’s toilet (sink and toilet bowl). Multiple swabs moving in different directions within approximately 25 cm² of surface area while rotating the swab stick were provided for each sampling area and stored in viral transport medium for transportation to the National Institute of Hygiene and Epidemiology (NIHE). In addition, checklists and questionnaires were used to collect information on disinfection practices in the study facilities.

2.2. Waste and wastewater sampling

Samples were collected from a garbage container (lid, inside garbage) in patient areas and hospital or quarantine centers. Regarding wastewater sampling, an approximately 1-liter sample grab was collected from the sewer line, pre- and post-treated tanks, then concentrated and extracted for SARS-CoV-2 testing after transporting them to the laboratory. The two-phase PEG-dextran method was applied for wastewater concentration and extraction (Deshpande and Siddiqui, 2020; Hovi et al., 2012; WHO, 2003).

2.3. Sample testing

All collected samples were transported in an ice-pack cool box to the NIHE for SARS-CoV-2 testing in 24 h. A real-time RT-PCR assay with the SuperScript III one-step RT-PCR system (Invitrogen, Carlsbad, CA, USA), targeting the E and RdRp genes following the WHO protocol is used to detect SARS-CoV-2 (WHO, 2020c). If detected by RT-PCR, then positive samples are subjected to cell culture to determine viability. As for virus isolation, Vero E6 cells, which are maintained in Eagle’s minimal essential medium containing 5% (v/v) newborn calf serum, are inoculated 100 μL of real-time RT-PCR-positive samples and incubated at 37 °C. The cultures were monitored daily for cytopathic effect. All experiments are to be performed in a biosafety level 3 (BSL-3) laboratory.

3. Results

A total of 468 environmental samples consisting of 43 inanimate surfaces, 30 garbage, and 25 wastewater samples were collected from both health care facilities and quarantined centers during August to October 2020 (Figs. 1 and 2). However, the real-time RT-PCR test results showed that all (100%) samples including all inanimate surfaces and wastewater samples were negative to SARS-CoV-2 neither at a cycle threshold value of under 38 as per Standard Operating Procedure nor above 38. As a result, no sample was referred to viral culture and isolation.

As for recent disinfection practices, chlorine-based products dissolved at a concentration of 0.1% (1000 ppm) in the general context or 0.5% (5000 ppm) for blood and body fluid spills are routinely wiped twice a day and at the discharge of patients in healthcare facilities. Similar disinfection process was applied in quarantined centers. Chlorine-based products were utilized for the disinfection of garbage by spraying them inside and outside the garbage containers and garbage surface prior to transport to the internal landfill. Except for a healthcare facility that treated wastewater using Anaerobic-Anoxic-Axyc (AAO) technology, the rest pour chloramine 0.5% into the wastewater tank once a week for sterilization purposes.

4. Discussion and conclusions

According to our findings, all 468 environmental samples collected from hospitals and isolation facilities were negative for the SARS-CoV-2 virus, which resulted in virus culture was not performed. To date, the existence of the SARS-CoV-2 virus on environmental surfaces in medical facilities still has mixed evidence. Several studies from different countries have reported detection of SARS-CoV-2 on surfaces in the patient areas, ranging from low to very high prevalence (Chin et al., 2020; Colaneri et al., 2020; Guo et al., 2020; Ong et al., 2020; Rajendiran et al., 2021; Tan et al., 2020).

While many studies have detected the presence of this virus on surfaces, some studies have not found its presence still. Research by Jie Wang et al. at the hospital affiliated to Zhejiang University confirmed that there was no presence of SARS-CoV-2 on the surface of doorknobs, gloves or the front of N95 mask wore by medical staff in the isolation area (Wang et al., 2020). The research by XN Yuan et al. at Peking University Hospital also found that 38 surface samples frequently exposed in contaminated areas (doorknobs, bed rails, control panels, faucets, etc.) and 16 surface samples of medical protective equipment (gloves, protective suits, etc.) were negative for SARS-CoV-2 (Yuan et al., 2020). The study by Kyeong Seob Shin et al. at the Chungbuk National Hospital concluded that the 12 surface samples in the treatment room of patients with positive SARS-CoV-2 were not detected for the presence of SARS-CoV-2 (Shin et al., 2020).
Even in healthcare facilities selected in our study that serve COVID-19 patients, the number of patients in these treatment facilities is not many, with a maximum of 24 patients at the time of sampling. The possibility of viral shedding was low at the sampling time, as most of the patients had asymptomatic or mild/moderate symptoms, even some of them had been treated for a few days (Muller, 2021; Murata et al., 2021; Sohn et al., 2020). There is uncertainty as to whether the situation affected our findings. However, no significant differences were found in positivity rates between studies with a different number of patients with COVID-19 (Gonçalves et al., 2021).

At the isolation facilities, there were no COVID-19 patients present at the time of sampling, but two of them recorded COVID-19 patients several days before. Then the patients had transferred to the hospital for treatment. In some studies in non-healthcare facilities, SAR-CoV-2 virus were detected on high-touch surfaces, none of SARS-CoV-2 was traced in our study (Ben-Shmuel et al., 2020; Luo et al., 2020).

In both healthcare facilities and quarantine centers, disinfection practices comply with WHO guidelines and are applied twice a day with chlorine-based products (WHO, 2020a, 2020b). Consequently, the appropriate practices may result in the elimination of the SAR-CoV-2 on surfaces and waste as chloramines are able to effectively kill pathogens such as bacteria, fungi, viruses, protozoa. Furthermore, hypochlorous acid (HOCl) induced from chlorine damage DNA, RNA, and polynucleotides (Dhamo et al., 2021; Hawkins and Davies, 2002; Prütz, 1996; United States Environmental Protection Agency). Given that an experiment suggested the impact of HOCl on primers and probes in the PCR system used for the detection of SARS-CoV-2, it is unlikely to be the case, as all facilities in our studies have followed the WHO guideline in the disinfection process. At each application, disinfectants were leaved on the surfaces for 10 min, then to be removed by clean water (Duan et al., 2021; WHO, 2020a).

Our study had some limitations. First, we did not measure ambient humidity and temperature, which can have an effect on virus survival at increased humidity and temperature (Biryukov et al., 2020; Riddell et al., 2020). However, the sampling time of our study was in a cool and dry period (end of summer and beginning of fall). Second, we did not collect samples on the following days to better understand the presence of the virus as the generic WHO protocol because of limited resources.

In summary, the finding may illustrate the importance of disinfection practices in removing pathogens or significantly reducing SARS-CoV-2 contamination on environmental surfaces and waste.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Le Thi Phuong Mai: Conceptualization, Writing – original draft. Van Dinh Tran: Formal analysis, Investigation. Hoang Vu Mai Phuong: Methodology, Validation. Ung Thi Hong Trang: Resources, Investigation. Le Thi Thanh: Resources. Nguyen Vu Son: Investigation. Vuong Duc Cuong: Visualization, Investigation. Luu Phuong Dung: Data curation. Nguyen Thi My Hanh: Project administration. Hoang Hai: Investigation. Dang Thi Kieu Oanh: Investigation. Nguyen Thanh Thuy: Writing – review & editing.

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Disclaimer

The funders played no role in the decision to conduct this study, the analysis, findings, or writing of this manuscript.

Ethical issue

Our study did not require an ethical board approval because it did not contain human or animal trials

Availability of data and materials

Data available on request from journal.
Advantages of biocidal sprays during the COVID-19 pandemic

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