Tropical Biomedicine 38(3): 462-468 (2021)
https://doi.org/10.47665/tb.38.3.089

RESEARCH ARTICLE

Environmental surface sampling of SARS-CoV-2 in selected hospitals in Malaysia

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ARTICLE HISTORY

Received: 6 August 2021
Revised: 10 September 2021
Accepted: 10 September 2021
Published: 30 September 2021

ABSTRACT

COVID-19 has spread rapidly worldwide. The role of fomites in facilitating onward transmission is plausible. This study aimed to determine the presence of viable virus and its persistence on the surfaces of fomites in wards treating COVID-19 patients in Malaysia. This study was conducted in two stages. First, environmental sampling was performed on random days in the intensive care unit (ICU) and general wards. Then, in the second stage, samples were collected serially on alternate days for 7 days in two selected general wards. In Stage 1, a total of 104 samples were collected from the surfaces of highly touched and used areas by patients and healthcare workers. Only three samples were tested positive for SARS-COV-2. In Stage 2, three surface samples were detected positive, but no persistence of the virus was observed. However, none of the SARS-CoV-2 RNA was viable through tissue culture. Overall, the environmental contamination of SARS-CoV-2 was low in this hospital setting. Hospitals' strict infection control and the compliance of patients with wearing masks may have played a role in these findings, suggesting adherence to those measures to reduce occupational exposure of COVID-19 in hospital settings.

Keywords: Environmental; surface sampling; SARS-CoV-2; Malaysia.

INTRODUCTION

The emerging coronavirus disease (COVID-19) infection is a global health crisis that burdens the healthcare system and affects the socioeconomic sectors. It was first detected in Wuhan, China from a cluster of patients with unknown causes of pneumonia in December 2019 and was provisionally named as 2019 novel coronavirus (2019-nCoV) (Zhu *et al*., 2019). The fast spread of this outbreak globally caused the inauguration of the Public Health Emergency of International Concern by the World Health Organization (WHO) on January 30, 2020. Later, the WHO declared coronavirus disease (COVID-19) the new name for this novel viral pneumonia on February 11, 2020 (WHO, 2020a) and the International Committee on Taxonomy of Viruses (ICTV) suggested the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on its phylogeny and taxonomy (Gorbalenya *et al*., 2020).

As of July 28, 2020, 16 341 920 COVID-19 cases had been reported globally resulting in 650 805 deaths (WHO, 2020b). Whereas in Malaysia, the first case of COVID-19 was reported on January 24, 2020. The first wave of infection comprised of 22 cases which were successfully handled (WHO, 2020c). However, there was a sudden surge of second wave of infection which begun from February 27, 2020 has resulted for a total of 8 904 cases and 124 deaths as of July 28, 2020 (Li *et al*., 2020; Shah *et al*., 2020; WHO, 2020d). Ministry of Health (MOH) Malaysia plays a pivotal role in containing the virus (Shah *et al*., 2020).

Human to human transmission has been established and relevant authorities are putting their effort on reducing the transmission (Li *et al*., 2020). However, the role of the environment in transmitting the virus is tenable (van Doremalen *et al*., 2020; WHO, 2020d). The involvement of contaminated surfaces is referred to as indirect contact transmission. Hand contact on contaminated fomites may facilitate onwards transmission through self-inoculation into the mucous membrane of the eyes, nose or mouth (Otter *et al*., 2016). When there was no definitive transmission

pathway could be established, and indirect transmission was suggested for the outbreak in a shopping mall cluster of COVID-19 cases in Wenzhou, China (Cai *et al*., 2020).

Viruses that are shed from an infected person into the environment may survive on surfaces. Based on previous studies on SARS/MERS-CoV, the environmental contamination and survival of these viruses on surfaces have been reported (Chen *et al*., 2004; Song *et al*., 2015; Bin *et al*., 2016). The survival of viruses on fomites can be affected by the temperature and humidity of the environment. In a favourable environment, SARS-CoV has been shown to survive for 2 weeks and can be inactivated by high temperatures and high humidity (Chan *et al*., 2011). Van Doremalen *et al*. (2020) found that the stability of both SARS-CoV-1 and -2 *in vitro* were similar. SARS-CoV-2 was stable at 4°C and on smooth surfaces and was sensitive to high temperatures (Chin *et al*., 2020). Its viability was longer on plastic and stainless-steel surfaces than on cardboard and copper (Chan *et al*., 2011, Chin *et al*., 2020).

Recently, several studies on SARS-CoV-2 succeeded in detecting viral RNA by PCR on environmental surfaces. However, most of the studies were only able to provide evidence of viral shedding and not viability of the virus (Cai *et al*., 2020; Chia *et al*., 2020; Colaneri *et al*., 2020; Ong *et al*., 2020; Su *et al*., 2020; Ye *et al*., 2020). Our study aimed to determine the environmental contamination of viable SARS-CoV-2 and its persistence on fomites in wards treating COVID-19 patients in Malaysia.

MATERIALS AND METHODS

Environmental surface sampling

Environmental Health Research Centre (EHRC) team of Institute for Medical Research (IMR) conducted an investigation on environmental surface sampling study from 25th March 2020 until 17th April 2020 in the intensive care units (ICUs) and general wards of two hospitals involved in the management of COVID-19 patients in Klang Valley, Malaysia. The samples were collected from highly touched and used areas by patients and healthcare workers. There were two stages of sampling.

Stage 1 was conducted as one-off sampling at the ICUs of hospital A (ICU X, ICU Y), general wards of hospital A (Isolation Room 1, Open Ward 4, Open Ward 5) and hospital B (Isolation Room 2, Isolation Room 3 and Open Ward 6). A total of 12 samples were taken from each general wards of both hospitals. Whereas 10 samples were collected from ICU X and 11 samples respectively from Isolation Room A and Isolation Room B of ICU Y. For Stage 2, environmental surface samples were collected serially every other day from the same sites of two different general wards in Hospital B. On every sampling day, ten samples were collected respectively from Open Ward S and Isolation Room T.

The sampling sites were selected based on the previous literature of highly touched surfaces of MERS-CoV and SARS-CoV as well as WHO's protocol on surface sampling of COVID-19 (Chen *et al*., 2004; Bin *et al*., 2016; van Doremalen *et al*., 2020). Samples were collected from patient's cubicle or room (doorknob, bedrail, pillowcase, side table, cardiac table, floor at 1 meter from patient's bed, ventilation outlet or window, blood pressure cuff, oximeter) and areas in toilet (sink and toilet bowl) of general wards for both stages. Whereas in ICU, samples were collected from patient's cubicle (doorknob, bedrail, bedsheet, side table, floor 1 meter from patient's bed, ventilation outlet, intravenous drip stand) and staff area (oximeter, monitor, phone, keyboard, mouse, soles of medical staff). The collection of samples was conducted in the morning before cleaning. The temperature and humidity were also recorded upon entering the respective area for sampling.

Environmental surface sampling was conducted in accordance with the WHO protocol. The investigators wore full personal protective equipment (PPE) during the collection of samples and complied with the respective hospital protocol on donning and doffing procedures. The surface samples were collected using sterile Dacron swabs pre-moistened with viral transport medium (VTM). Then, the swabs were kept into a tube containing 1.5 mL of VTM solution. Each tube was labelled and kept in two layers of sealed biohazard plastic bags. The samples were transported to the laboratory at 4°C. Field control was also performed by opening a swab and directly inserting it into a tube containing VTM without sampling any surfaces on each sampling day.

Sample Lysis and Extraction

At the laboratory, the samples were aliquoted for real-time reverse transcription (RT) polymerase chain reaction (PCR) analysis and culture. For PCR analysis, the samples were subjected to heat inactivation at 65°C for an hour, followed by viral RNA extraction using the QIAamp Viral RNA Mini Kit (QIAGEN, USA) according to the manufacturer's instructions (Qiagen, 2020). A volume of 5 µL of the extract was used for analyses. SARS-CoV-2 RNA detection in the samples was performed using real time RT-PCR assay targeting the RNA-dependent RNA polymerase (RdRP) gene on the Bio-Rad CFX-96 platform as described (Corman *et al*., 2020).

Viability of the Virus

Positive samples by RT-PCR were then subjected to cell culture to determine viability. Cell culture was performed in a biosafety level 3 (BSL-3) laboratory. Vero E6 cells were grown overnight in Hanks' minimum essential medium (HMEM) (Sigma-Aldrich, USA) supplemented with 10% foetal bovine serum (FBS) (Gibco, USA) in 15 mL Corning® culture tubes (Sigma-Aldrich, USA). To culture the SARS-CoV-2 virus, 200 µL of each environmental specimen in VTM was added to the Vero E6 cells and incubated for an hour at room temperature. The infected cells were then maintained with 1 mL of HMEM supplemented with 2% FBS and monitored for CPE. Next, the infected culture tubes were frozen at -80°C overnight and then thawed. The culture supernatants were filtered and also tested for the growth of SARS-CoV-2 by determining the cycle threshold (Ct) value using real-time RT-PCR.

RESULTS

Stage 1 of the Environmental Surface Sampling

There was no surface contamination detected in any of the ICUs, Isolation Room 1 or Isolation Room 2. However, in Open Ward 5, the surface sample was positive for SARS-CoV-2 at the cardiac table (1/12, 8.33%) with a Ct value of 37.01. Other areas of SARS-CoV-2 detection were at the sinks in Isolation Room 3 (1/12, 8.33%) and Open Ward 6 (1/12, 8.33%), with Ct values of 34.43 and 37.52, respectively. However, no growth was seen when the samples were cultured. The environmental surface contamination for Stage 1 in relation to occupancy of patients, types of ventilation, temperature and humidity of respective sampling location is shown in Figure 1.

Stage 2 of the Environmental Surface Sampling

In Open Ward S, only the sample taken from the floor was positive for SARS-CoV-2 on the first day of sampling (1/10,

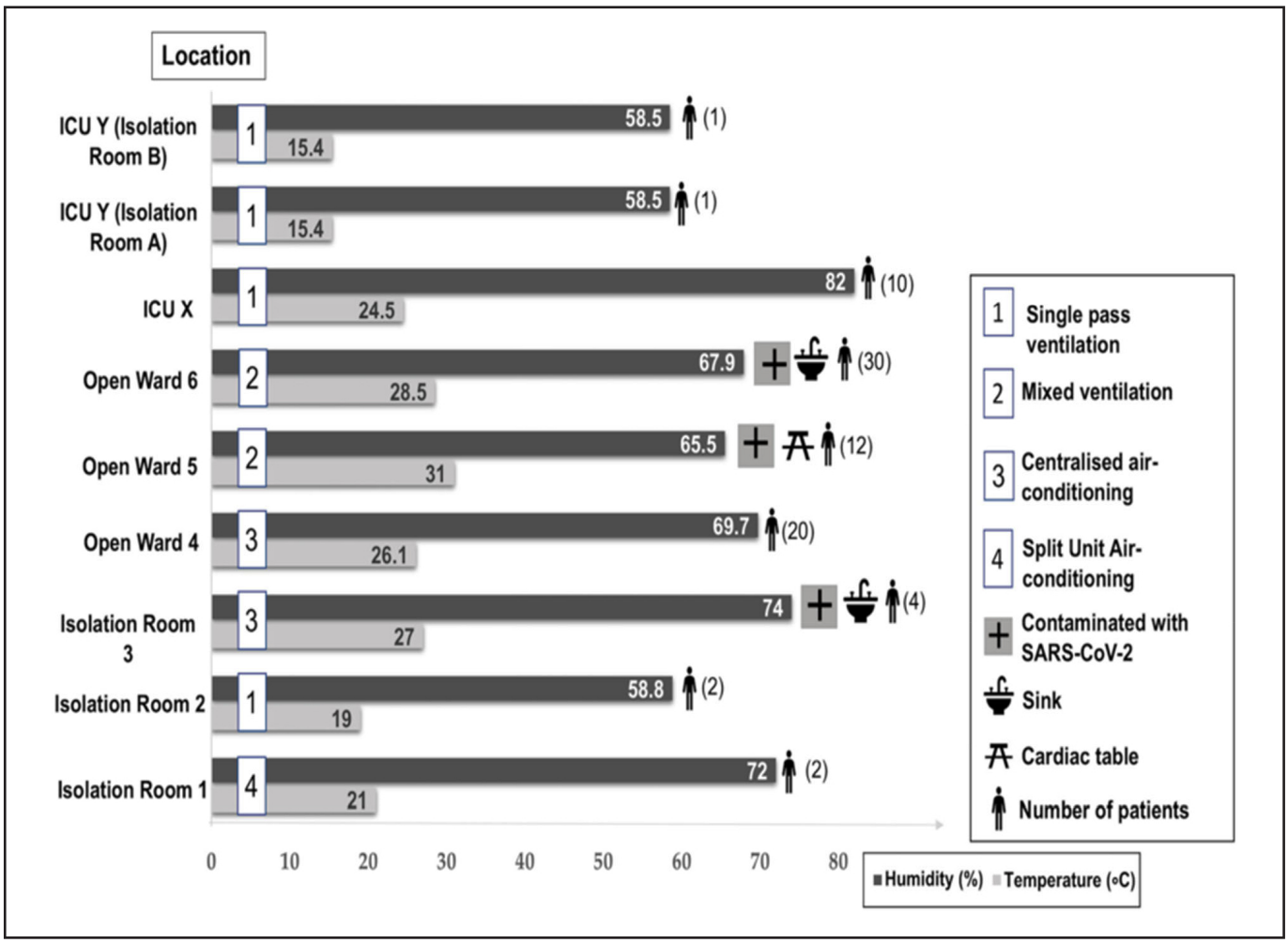

Figure 1. Detection of SARS-COV-2 among environmental surface samples for Stage 1. The site of samples which showed positive for SARS-CoV-2 was depicted through symbol. The details on occupancy of patients, types of ventilation, temperature and humidity of respective sampling locations were included.

10%, with a Ct value of 37.01). On day 3, 2 samples (2/10, 20%) were positive for SARS-CoV-2 from the sink and toilet bowl, with Ct values of 35.59 and 33.49, respectively. On subsequent sampling days, SARS-CoV-2 was not detected at any of the sites. All the samples detected positive for SARS-CoV-2 by PCR were unable to grow in cell culture. The ward had mixed ventilation of the ceiling fan and a mobile air conditioning unit. In Isolation Room T, SARS-CoV-2 was not detected throughout the serial sampling. The room had split unit air conditioning. Figure 2 shows the environmental surface contamination for Stage 2 in relation to occupancy of patients, temperature and humidity of respective sampling location.

General Wards for Both Stages

Overall, the detection of SARS-CoV-2 was higher for sites in the toilet than for sites close to the patient's bed. In the patient area, two surface samples out of 124 (1.61%) were contaminated with SARS-CoV-2, and 4 out of 28 samples (14.3%) were contaminated at the toilet area. The positivity rates for the sink and toilet bowl were 21.4% (3/14) and 7.14% (1/14), respectively.

DISCUSSION

Contaminated fomite in hospital setting may facilitate onwards transmission of the virus to healthcare workers. Furthermore, a few studies from different countries have reported detection of SARS-CoV-2 in areas housing COVID-19 patients which were variable and ranged from 2.8-87% (Chia *et al*., 2020; Colaneri *et al*., 2020; Guo *et al*., 2020; Ong *et al*., 2020; Ye *et al*., 2020). Although a study from Wuhan, China, reported high positive rate of SARS-CoV-2 being detected from surface samples in hospital wards (Guo *et al*., 2020), our rate of detection was quite low (3.2%) which was similar to the findings by Tan *et al*. (2020).

Ministry of Health, Malaysia came up with a guideline on infection prevention and control (IPC) measures very early during COVID-19 outbreak which was last updated in March 2020. Among the measures in the guideline include regular disinfecting ward surfaces especially those highly touched areas by patients and mopping the floor at least twice daily using a 2.5 g effervescent chlorinated tablet, which was dissolved into 1 litre of water; changing of disposable bedsheets and pillowcases daily and upon discharge and usage of disposable food container and utensils (Ministry of Health Malaysia, 2020). Strict adherence to this IPC measures may have attributed to the low detection rate which was also suggested by Tan *et al*. (2020).

Apart from that, patients were provided with masks to wear at all times during their hospital stay. It was observed during our sampling that they were compliant to it continuously except during meals and drinking water. Since mask can prevent expulsion of the virus, removal of mask may cause shedding of the virus into environment. Generally,

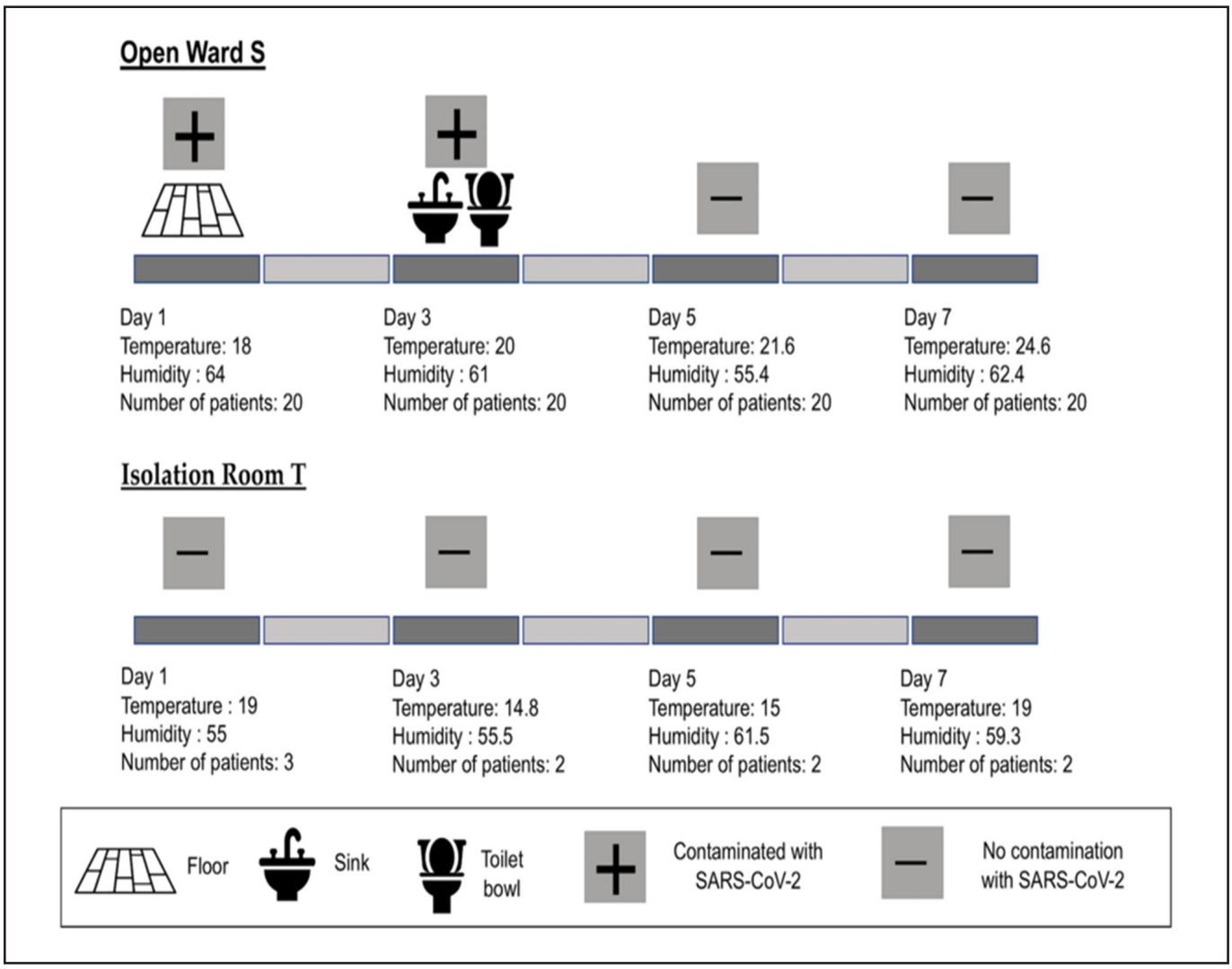

Figure 2. Detection of SARS-COV-2 among environmental surface samples for Stage 2. The site of samples which showed positive for SARS-CoV-2 was depicted through symbol. The details on occupancy of patients, temperature and humidity of respective sampling locations were included.

respiratory droplets will settle down on nearby surfaces or floor due to gravity pull (Jayaweera *et al*., 2020). In our study, we detected SARS-CoV-2 RNA only in one sample each from patient's cardiac table and floor. This was detected in between two cleaning periods and the virus could have probably been shed into the environment when patient was eating, coughing or sneezing.

However, contamination floor surfaces with the virus can become a potential pathway of transmission from healthcare worker to community. In accordance with this, healthcare workers can carry the virus when they walk around and smearing it at other areas, as it been observed in pharmacy, a place that not accessed by COVID-19 patients, recorded high positive rate for floor swabs (Tan *et al*., 2020). Hence, the base of shoes of healthcare workers act as carrier for this virus. Our limitation was that we didn't perform surface samples of soles of medical staff in general wards. When we tested it in ICU, there was no detection of the virus.

Common routes of excretion of SARS-CoV-2 are through saliva and faeces (Jayaweera *et al*., 2020; McDermott *et al*., 2020). Wang *et al*. (2020) found 29% positivity rate in faeces of 153 COVID-19 patients. Studies reported that shedding of the virus continued for a median duration of 22 days irrespective of patient's severity of the disease (Zheng *et al*., 2020) and the viral load is usually higher in saliva in early phase of COVID-19 infection which can persist up to 11 days (To *et al*., 2020). In our hospital setting, toilets were shared by patients in respective rooms or wards which may have led to the higher contamination rate in this area compared to individual patient's surrounding area in present study. This may explain the higher detection rate of SARS-CoV-2 RNA from sinks and toilet bowls in our study. Similar finding was reported by Ong *et al*. (2020) and Santarpia *et al*. (2020).

Notably, studies found ICU as one of the highly contagious area due to aerosol generating procedures like endotracheal intubation, suctioning and nebulization (Jayaweera *et al*., 2020). This was further corroborated by detection of the virus in air samples taken near a patient who underwent endotracheal intubation. It was also reported that highly touched surfaces by severe or critical ill patients were highly contaminated compared to areas surrounding mild patients (Guo *et al*., 2020; Tan *et al*., 2020). On the contrary, we did not find any environmental contamination of SARS-CoV-2 at surfaces sampled in ICU despite accommodating severe COVID-19 patients with assisted ventilation. Similar finding was also reported by Chia *et al*. (2020).

It was found that there is a possible shift of the viral load level from oral swabs during the early phase of infection to anal swabs at a later stage (Pastorino *et al*., 2020). Upon sampling, it was observed that the patients in the ICU were mostly on prolonged stays and beyond 14 days of illness. However, a study has found that environmental contamination in areas housing COVID-19 patients' is irrespective to the days of illness where positive surface samples were even found from areas of patients on day 48 of illness (Tan *et al*., 2020). It was conjectured that the reason for the low environmental contamination could be due to restricted movement of patient as they were bed-ridden and adherence to infection measures by ICU staffs.

Studies have demonstrated that SARS-CoV-2 can persist up to a few days (Chin *et al*., 2020; Pastorino *et al*., 2020). Therefore, we performed serial environmental sampling to determine persistency of the virus in hospital setting. However, we were able to detect three positive sample on different surfaces on different days, there was no persistency of the virus was observed at the same sites. This is probably attributed by the IPC measures taken. This was supported by laboratory and field studies which demonstrated that this virus can be eliminated through standard disinfection and routine cleaning (Chan *et al*., 2020; Chin *et al*., 2020; Ong *et al*., 2020).

Although there were extensive studies on environmental surface sampling conducted in different countries but most of it did not perform cell culture to determine its viability (Chia *et al*., 2020; Colaneri *et al*., 2020; Guo *et al*., 2020; Ong *et al*., 2020; Ye *et al*., 2020) and only few studies have tested it in-vitro (Chan *et al*., 2020; Chin *et al*., 2020; van Doremalen *et al*., 2020). We did not culture all of the environmental samples taken and only restricted it to samples which were detected positive by RT-PCR due to time constraint and limited resources. The positive samples showed no growth by cell culture. Our findings may suggest that the risk of onwards transmission to healthcare workers is low.

It was reported Ct value of a sample can influence the infectivity of the virus in a cell culture model and demonstrated on the decrement of culture positivity rate as Ct values increases (La Scola *et al*., 2020). However, the specificity and sensitivity of environmental samples may differ from clinical samples. In present study, the positive samples had Ct values ranging between 33.49 to 37.52 and the Ct value of the culture supernatant remain unchanged. Exponential decay in titre of the virus over time in an *in-vitro* study was reported (van Doremalen *et al*., 2020). The viability of the surface samples can be affected by the different environmental conditions like temperature, pH and disinfectants (Biryukov *et al*., 2020; Chan *et al*., 2020; Chin *et al*., 2020).

The strength of this study was that we performed viability testing of the virus and also investigated the persistence of the virus through serial sampling. This present study involves two centres containing COVID-19 patient which complies to IPC guidelines set by MOH Malaysia; therefore, the results can be generalised to other healthcare facilities in our country. Furthermore, sampling in areas with different ventilation systems can be representative of community settings as well.

We are aware of our limitation in this study. First, we were limited in retrieving the patient's information on the day of illness and their viral load. It will be difficult to correlate environmental sampling with patient's information in view of the wards or rooms and toilets were shared by few patients in midst of outbreak. Besides that, there was no air sampling was performed. Our study focussed on surface sampling as a representative for the environmental contamination in hospital settings. In future studies, these limitations could be considered as to correlate those factors in relation to environmental contamination in such settings.

CONCLUSION

Overall, the environmental contamination rate of SARS-CoV-2 was low. This may be attributed to the good infection control of the hospitals. Moreover, the nonviability of the virus may not facilitate onwards transmission. Hence, frequent and thorough cleaning in hospital settings and the compliance of patients wearing masks are imperative to reduce the spread of this virus and to protect healthcare workers. However, in community settings, environmental contamination may be higher, and it has been suggested that individuals should practice frequent hand cleaning and wear masks to reduce the risk of contamination.

ACKNOWLEDGEMENTS

The authors are grateful to our Director-General Health of Malaysia for his permission to publish this article. In addition, the authors would like to thank the Deputy Director General of Health (research and technical support) and Director of Institute of Medical Research (IMR) for their support as well as those frontliners for COVID-19 who assisted us with our field work, the staff in EHRC and the Virology Unit who contributed to the success of this investigation. We would like to acknowledge the external reviewer of this paper as well. This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Bin, S.Y., Heo, J.Y., Song, M.S., Lee, J., Kim, E.H., Park, S.J., Kwon, H., Kim, S.M.I., Kim, Y., Si, Y.J. *et al*. (2016). Environmental contamination and viral shedding in MERS patients during MERS-CoV outbreak in South Korea. *Clinical Infectious Diseases* **62**: 755-760. https://doi.org/10.1093/cid/civ1020

Biryukov, J., Boydston, J.A., Dunning, R.A., Yeager, J.J., Wood, S., Reese, A.L., Ferris, A., Miller, D., Weaver, W., Zeitouni, N.E. A. *et al*. (2020) Increasing temperature and relative humidity accelerates inactivation of SARS-CoV-2 on surfaces. *mSphere* **5**: 1-9. https://doi.org/10.1128/mSphere.00441-20

Cai, J., Sun, W., Huang, J., Gamber, M., Wu, J. & He, G. (2020). Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020. *Emerging Infectious Diseases journal* **26**: 1343-1345. https://doi.org/10.3201/eid2606.200412

Chan, K.H., Peiris, J.S.M., Lam, S.Y., Poon, L.L.M., Yuen, K.Y. & Seto, W.H. (2011). The effects of temperature and relative humidity on the viability of the SARS coronavirus. *Advances in Virology* **2011**: 734690. https://doi.org/10.1155/2011/734690

Chan, K.H., Sridhar, S., Zhang, R.R., Chu, H., Fung, A.Y.F., Chan, G., Chan, J.F.W., To, K.K.W., Hung, I.F.N., Cheng, V.C.C. *et al*. (2020). Factors affecting stability and infectivity of SARS-CoV-2. *Journal of Hospital Infection* **106**: 226-231. https://doi.org/10.1016/j.jhin.2020.07.009

Chen, Y.C., Huang, L.M., Chan, C.C., Su, C.P., Chang, S.C., Chang, Y.Y., Chen, M.L., Hung, C.C., Chen, W.J., Lin, F.Y. *et al*. (2004). SARS in hospital emergency room. *Emerging Infectious Diseases Journal* **10**: 782-788. https://doi.org/10.3201/eid1005.030579

Chia, P.Y., Coleman, K.K., Tan, Y.K., Ong, S.W.X., Gum, M., Lau, S.K., Sutjipto, S., Lee, P.H., Son, T.T., Young, B.E. *et al*. (2020). Detection of air and surface contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in hospital rooms of infected patients. Infectious Diseases (except HIV/AIDS). *Nature Communications* **11**: 2800. https://doi.org/10.1038/s41467-020-16670-2

Chin, A.W.H., Chu, J.T.S., Perera, M.R.A., Hui, K.P.Y., Yen, H.L., Chan, M.C.W., Peiris, M. & Poon, L.L.M. (2020). Stability of SARS-CoV-2 in different environmental conditions. *The Lancet Microbe* **1**: e10. https://doi.org/10.1016/S2666-5247(20)30003-3

Colaneri, M., Seminari, E., Piralla, A., Zuccaro, V., Filippo, A.D., Baldanti, F., Bruno, R., Mondelli, M.U., Bruno, R., Mondelli, M.U. *et al*. (2020). Lack of SARS-CoV-2 RNA environmental contamination in a tertiary referral hospital for infectious diseases in Northern Italy. *Journal of Hospital Infection* **105**: 474-476. https://doi.org/10.1016/j.jhin.2020.03.018

Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T., Brünink, S., Schneider, J., Schmidtm, M.L. *et al*. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance* **25**: 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Gorbalenya, A.E., Baker, S.C., Baric, R.S., de Groot, R.J., Drosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., Leontovich, A.M., Neuman, B.W. *et al*. (2020). The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology* **5**: 536-544. https://doi.org/10.1038/s41564-020-0695-z

Guo, Z.D., Wang, Z.Y., Zhang, S.F., Li, X., Li, L., Li, C., Cui, Y., Fu, R.B., Dong, Y.Z., Chi, X.Y. *et al*. (2020). Aerosol and surface distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in hospital wards, Wuhan, China, 2020. *Emerging Infectious Diseases journal* **26**: 1583-1591. https://doi.org/10.3201/eid2607.200885

Jayaweera, M., Perera, H., Gunawardana, B. & Manatunge, J. (2020). Transmission of COVID-19 virus by droplets and aerosols: A critical review on the unresolved dichotomy. *Environmental Research* **188**: 109819. https://doi.org/10.1016/j.envres.2020.109819

La Scola, B., Le Bideau, M., Andreani, J., Hoang, V.T., Grimaldier, C., Colson, P., Gautret, P. & Raoult, D. (2020). Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. *European Journal of Clinical Microbiology & Infectious Diseases* **39**: 1059-1061. https://doi.org/10.1007/s10096-020-03913-9

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K.S.M., Lau, E.H.Y., Wong, J.Y. *et al*. (2020). Early transmission dynamics in Wuhan, China, of Novel Coronavirus – Infected Pneumonia. *The New England Journal of Medicine* **382**: 1199-1207. https://doi.org/10.1056/NEJMoa2001316

McDermott, C.V., Alicic, R.Z., Harden, N., Cox, E.J. & Scanlan, J.M. (2020). Put a lid on it: are faecal bio-aerosols a route of transmission for SARS-CoV-2? *Journal of Hospital Infection* **105**: 397-398. https://doi.org/10.1016/j.jhin.2020.04.024

Ministry of Health Malaysia (2020). Guidelines COVID-19 Management No.5/2020. http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm/Annex_8__IPC_21032020.pdf. Accessed 20 July 2020.

Ong, S.W.X., Tan, Y.K., Chia, P.Y., Lee, T.H., Ng, O.T., Wong, M.S.Y. & Marimuthu, K. (2020). Air, surface environmental, and personal protective equipment contamination by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *Journal of the American Medical Association* **323**: 1610-1612. https://doi.org/10.1001/jama.2020.3227

Otter, J.A., Donskey, C., Yezli, S., Douthwaite, S., Goldenberg, S.D. & Weber, D.J. (2016). Transmission of SARS and MERS coronaviruses and influenza virus in healthcare settings: the possible role of dry surface contamination. *Journal of Hospital Infection* **92**: 235-250. https://doi.org/10.1016/j.jhin.2015.08.027

Pastorino, B., Touret, F., Gilles, M., de Lamballerie, X. & Charrel, R.N. (2020). Prolonged infectivity of SARS-CoV-2 in fomites. *Emerging Infectious Diseases Journal* **26**: 2256-2257. https://doi.org/10.3201/eid2609.201788

Qiagen (2020). QiAamp Viral RNA Mini Kit. https://www.qiagen.com/my/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-mini-kit/?clear=true#orderinginformation. Accessed 18 March 2020.

Santarpia, J.L., Rivera, D.N., Herrera, V., Morwitzer, M.J., Creager, H., Santarpia, G.W., Crown, K.K., Brett-Major, D., Schnaubelt, E., Broadhurst, M.J. *et al*. (2020). Aerosol and surface transmission potential of SARS-CoV-2. *Scientific Reports* **10**: 12732. https://doi.org/10.1038/s41598-020-69286-3

Shah, A.U.M., Safri, S.N.A., Thevadas, R., Noordin, N.K., Rahman, A.A., Sekawi, Z., Ideris, A. & Sultan, M.T.H. (2020). COVID-19 outbreak in Malaysia: Actions taken by the Malaysian government. *International Journal of Infectious Diseases* **97**: 108-116. https://doi.org/10.1016/j.ijid.2020.05.093

Song, J.Y., Cheong, H.J., Choi, M.J., Jeon, J.H., Kang, S.H., Jeong, E.J., Yoon, J.G., Lee, S.N., Kim, S.R., Noh, J.Y. *et al*. (2015). Viral shedding and environmental cleaning in Middle East Respiratory Syndrome Coronavirus Infection. *Infection & Chemotherapy* **47**: 252. https://doi.org/10.3947/ic.2015.47.4.252

Su, W.L., Hung, P.P., Lin, C.P., Chen, L.K., Lan, C.C., Yang, M.C., Peng, M.Y. & Chao, Y.C. (2020). Masks and closed-loop ventilators prevent environmental contamination by COVID-19 patients in negative-pressure environments. *Journal of Microbiology, Immunology and Infection* **54**: 81-84. https://doi.org/10.1016/j.jmii.2020.05.002

Tan, L., Ma, B., Lai, X., Han, L., Cao, P., Zhang, J., Fu, J., Zhou, Q., Wei, S., Wang, Z. *et al*. (2020). Air and surface contamination by SARS-CoV-2 virus in a tertiary hospital in Wuhan, China. *International Journal of Infectious Diseases* **99**: 3-7. https://doi.org/10.1016/j.ijid.2020.07.027

To, K.K.W., Tsang, O.T.Y., Yip, C.C.Y., Chan, K.H., Wu, T.C., Chan, J.M.C., Leung, W.S., Chik, T.S.H., Choi, C.Y.C., Kandamby, D.H. *et al*. (2020). Consistent detection of 2019 Novel Coronavirus in saliva. *Clinical Infectious Diseases* **71**: 841-843. https://doi.org/10.1093/cid/ciaa149

van Doremalen, N., Bushmaker, T., Morris, D.H., Holbrook, M.G., Gamble, A., Williamson, B.N., Tamin, A., Harcourt, J.L., Thornburg, N.J., Gerber, S.I. *et al*. (2020). Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *The New England Journal of Medicine* **382**: 1564-1567. https://doi.org/10.1056/NEJMc2004973

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G. & Tan, W. (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *Journal of the American Medical Association* **323**: 1843-1844. https://doi.org/10.1001/jama.2020.3786

World Health Organization (2020a). Archived: WHO Timeline-COVID-19. https://www.who.int/news-room/detail/27-04-2020-who-timeline—covid-19. Accessed 25 May 2020.

World Health Organization (2020b). Coronavirus disease (COVID-19) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed 29 July 2020.

World Health Organization (2020c). COVID-19 situation overview in Malaysia. https://www.who.int/docs/default-source/wpro—documents/countries/malaysia/coronavirus-disease-(covid-19)-situation-reports-in-malaysia/situation-report-malaysia-11-july-2020.pdf?sfvrsn=5fb63c93_6. Accessed 28 September 28 2020.

World Health Organisation (2020d). Surface sampling of coronavirus disease (COVID-19): a practical "how to" protocol for health care and public health professionals, version 1. https://apps.who.int/iris/handle/10665/331058. Accessed 17 March 2020.

Ye, G., Lin, H., Chen, S., Wang, S., Zeng, Z., Wang, W., Zhang, S., Rebmann, T., Li, Y., Pan, Z. *et al*. (2020). Environmental contamination of SARS-CoV-2 in healthcare premises. *Journal of Infection* **81**: e1-5. https://doi.org/10.1016/j.jinf.2020.04.034

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X. *et al*. (2020). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *British Medical Journal* **360**: m1443. https://doi.org/10.1136/bmj.m1443

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. *et al*. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *The New England Journal of Medicine* **382**: 727-733. https://doi.org/10.1056/NEJMoa2001017