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Short Communication

Masks and closed-loop ventilators prevent environmental contamination by COVID-19 patients in negative-pressure environments

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Abstract Herein, we report that nosocomial infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may be mitigated by using surgical masks and closed looped ventilation for both non-critical and critical patients. These preventive measures resulted in no viral contamination of surfaces in negative pressure environments.

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Introduction

In early 2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread from China to the rest of the world, with most countries reporting nosocomial infections by the end of March.1–3 Recently, a study conducted in Singapore has explored the mode of transmission and
extent of environmental contamination in ambulatory patients. A concurrent study revealed that turbulent gas clouds around critical patients intubated with mechanical ventilators increased the risk of viral transmission. While SARS-CoV-2 can be detected on stainless steel or plastic for at least 2–3 days after initial contamination, the actual viral load on surfaces in negative-pressure intensive care units (ICU) remains unknown. In this study, we examine viral contamination by SARS-CoV-2 positive patients on surfaces in the ICU and an ordinary ward for 9 days.

Methods

The local surfaces around three patients were tested for viral contamination. Patient 1 was an 85-year-old male patient with critically severe SARS-CoV-2 pneumonia exhibiting respiratory failure and requiring mechanical ventilation. Patient 2, a 68-year-old male, and Patient 3, a 60-year-old female, both presented simple SARS-CoV-2 bronchopneumonia. All three patients were admitted to a negative-pressure isolation room in either a medical ICU (Patient 1) or an ordinary ward with 12 air exchanges per hour (patients 2 and 3). The negative-pressure differences for the ward area to buffer area and clean corridor to buffer area were −14 to −15 Pa and −8 to −9 Pa, respectively. The ward temperature was 23.2–23.9 °C and the relative humidity was 58.3–59.7%. For Patient 1, a heat and moisture exchange (HME) filter (VH-3110 FHME, Great Group Medical CO., LTD., Taiwan) was installed between the tubing loop from the patient to the ventilator to prevent the virus entering the device. A closed suction device (T20005 PAHSCO, Pacific Hospital Supply CO., LTD., Taiwan) was also connected for sputum suction and to prevent viral transmission. A nebulizer device was omitted from this study to limit aerosolized contamination. For the two non-critical patients, surgical masks were redressed daily. All areas were cleaned daily with a 1:10 dilution of 5% sodium hypochlorite, except the floor where a dilution ratio of 1:100 was used.

For Patient 1 in the ICU, environmental samples were collected from 16 sites (Figure S1) before routine cleaning on the same dates as throat swabs, peritoneal dialysate, and anal swabs were collected (March 9, 12, and 18, 2020). Sterile throat swabs (LIBO Specimen Collection and Transport Swabs, LIBO Medical Products Inc., Taiwan) were used for all environmental sampling and were transported in a Viral Transport Medium (CMP®, Taiwan). For the non-critical patients, environmental and clinical samples were collected from the 15 relative sites before routine cleaning on the same dates as positive throat swabs (April 2, 6, and 8, 2020). The percentage of positive results was verified through triplicate sampling at each site.

All samples were analyzed for SARS-CoV-2 using reverse transcriptase-polymerase chain reaction (RT-PCR) detection. Specific RT-PCR targeting of RNA-dependent RNA polymerase (RdRp), E, and N genes were used to detect the presence of SARS-CoV-2. The number of cycles required for the fluorescent signal to cross the threshold in RT-PCR (cycle threshold, Ct values) determines the viral load with lower Ct values corresponding to higher viral loads. Samples were sent to a biosafety level 2 (BSL-2) laboratory with a built-in negative-pressure room for immediate storage at −20 °C prior to RT-PCR testing, which was conducted within three days of sampling. For the RdRp gene, a 20 µl reaction was performed with 5 µl of template RNA and 15 µl of the PCR reaction master mix, as suggested by LightMix® Modular SARS-CoV-2 (COVID19) RdRP, and samples were detected on a Roche z480 (Roche Molecular Systems, Inc., California, US). Thermal cycling was performed at 55 °C for reverse transcription followed by initial denaturation at 95 °C for 5 min then 45 cycles of amplification at 95 °C for 5 s, 60 °C for 15 s, and 72 °C for 15 s, followed by cooling at 40 °C for 30 s. The positive findings were defined as the least significant Ct of the E and RdRP genes.

Results

Positive throat swabs for SARS-CoV-2 were obtained on three separate dates for all three patients. The corresponding environmental and clinical sample data are shown in Table 1 and Figure S1. For Patient 1, the throat swab exhibited positive RT-PCR E gene Ct values of 26.73, 28.85, and 29.86 on March 9, 12, and 18, 2020, respectively. Before routine cleaning, all environmental samples of the ICU were negative except for “Ventilator tubing before HME filter,” which was strongly positive, with an E gene Ct value of 30.41 on March 9, 2020 and 31.79 on March 12, 2020. Similarly, all clinical data exhibited negative RT-PCR results except for the closed suction tube specimen, which had Ct values of 29.52, 30.64, and 33.07 on March 9, 12, and 18, 2020, respectively.

The throat swabs from Patient 2 exhibited positive RT-PCR results with E gene Ct values of 23.46, 28.97, and 27.77 on April 2, 6, and 8, 2020, respectively. The E gene Ct values of 26.83, 28.37, and 29.31 on April 2, 6, and 8, 2020, respectively, were detected for non-critical Patient 3. In the same situation, before routine cleaning, all environmental samples of the ordinary wards were negative except for “surgical mask,” which was weakly positive, with Ct values of 33.62, 36.24 on April 2, 2020 and 34.82, 37.35 on April 6, 2020.

Discussion

Viral load was undetectable with a daily routine cleaning using standard sodium hypochlorite, regardless if it was the ICU or an ordinary ward. These results contrast with those of Ong et al., which might be explained by two critical differences in testing. First, the patient in the ICU was bedridden; thus, he was unable to contaminate the ward environment. The virus present in the patient’s phlegm was contained by the closed suction tube and the HME filter with closed-loop ventilation. The negative anal swab and peritoneal fluid also indicated no possibility of contamination from the toilet.

Similarly, the surgical masks worn by patients 2 and 3 at all times, except when eating, contained the virus. Second, the medical team treating all three patients wore personal protective equipment when entering the negative-pressure ward, as well as two to three sets of gloves. The outermost gloves were changed or washed with 75% alcohol after performing the upper airway procedure or
Environmental samples were collected around the patient where contamination may have been induced by the medical team. Clinical data specimens were collected from potential virus transmission routes near the patient. Calculated number of positive results out of three examinations on separate dates.

Environmental samples were collected around the patient where contamination may have been induced by patients themselves.

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### Table 1

| Sites                              | Patient 1 | Sites                              | Patient 2 | Patient 3 |
|------------------------------------|-----------|------------------------------------|-----------|-----------|
| Environmental sitesa               | Positive ratec | Environmental sitesd               | Positive ratec | Positive ratec |
| 1. Control panel on bed            | 0/3       | 1. Control panel on bed            | 0/3       | 0/3       |
| 2. Entire length of bed rail       | 0/3       | 2. Entire length of bed rail       | 0/3       | 0/3       |
| 3. Mattress                        | 0/3       | 3. Mattress                        | 0/3       | 0/3       |
| 4. Locker with hand slot          | 0/3       | 4. Locker with hand slot          | 0/3       | 0/3       |
| 5. Vital sign monitor panel button | 0/3       | 5. Light switch                    | 0/3       | 0/3       |
| 6. Ventilator tubing before HME filter | 2/3 | 6. Pillowcase                      | 0/3       | 0/3       |
| 7. Ventilator tubing after HME filter | 0/3 | 7. Curtain                        | 0/3       | 0/3       |
| 8. Ventilator panel button         | 0/3       | 8. Kettle handle                   | 0/3       | 0/3       |
| 9. Infusion pump panel button      | 0/3       | 9. Infusion stand                  | 0/3       | 0/3       |
| 10. Toilet door handle            | 0/3       | 10. Toilet door handle             | 0/3       | 0/3       |
| 11. Toilet faucet handle          | 0/3       | 11. Toilet faucet handle          | 0/3       | 0/3       |
| 12. Closed suction tube            | 3/3       | 12. Surgical Mask                  | 2/3       | 2/3       |
| 13. Peritoneal dialysate           | 0/3       | 13. Gargling fluid                | 0/3       | 0/3       |
| 14. Anal swab                      | 0/3       |                                     |           |           |

a Environmental samples were collected around the patient where contamination may have been induced by the medical team.
b Clinical data specimens were collected from potential virus transmission routes near the patient.
c Calculated number of positive results out of three examinations on separate dates.
d Environmental samples were collected around the patient where contamination may have been induced by patients themselves.

Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; HME, heat and moisture exchange; HEPA, high-efficiency particulate absorption.
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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2020.05.002.