Airborne Severe Acute Respiratory Syndrome Coronavirus Concentrations in a Negative-Pressure Isolation Room

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This study used a sensitive polymerase chain reaction method coupled with filter sampling to detect the presence of airborne severe acute respiratory syndrome (SARS) coronavirus in an isolation patient room with a patient with severe acute respiratory syndrome receiving mechanical ventilatory support. Polymerase chain reaction results were negative for SARS coronavirus in room air both before and after patient extubation.

Severe acute respiratory syndrome (SARS) is a respiratory infectious disease that has been reported in Asia, North America, and Europe.[1] So far 8,096 probable SARS cases and 774 deaths have been reported, giving a worldwide case fatality rate of 9.6%.[2] Infectious agents are carried in airborne droplets produced by aerosolization that can occur from sneezing, coughing, and talking.[3] Most droplets are 4-8 μm in diameter, by microscopic measurement.[4] Aerosolized SARS coronavirus (CoV) droplets range in size from 0.1 to 0.2 μm[1,5] and survive in the ambient environment for a couple of days.[6]

The World Health Organization has indicated that air travelers seated within 2 rows of an infected person could be in danger of contracting SARS-CoV.[7] Attack rates for hospital workers are associated with the number of SARS patients admitted to the individual hospitals.[8] Preventive measures, such as wearing N95 masks and hand washing, are effective for avoiding contact with the respiratory secretions of SARS patients.[9] Flow balancing of the air distribution system in hospital wards should be performed once a year to decrease nosocomial transmission.[10]

Until recently, few studies had sampled the virus-containing aerosols generated during expulsions from the patient's respiratory tract. Polymerase chain reaction (PCR) analysis amplifies nucleic acids exponentially and is particularly sensitive to the detection of infectious agents.[11-14] We therefore specifically evaluated airborne SARS-CoV DNA concentrations in a negative-pressure isolation room that housed a patient with SARS who was receiving mechanical ventilatory support. We also evaluated the filtration efficiency of the high-efficiency particulate air (HEPA) filters connected to the exhaled breathing circuit of the mechanical ventilator.

METHODS

Patient. A female patient with SARS who had throat and nasopharynx samples that tested positive by PCR for SARS-CoV on May 12, 2003, was housed in a negative-pressure room in the Chang Gung Memorial Hospital (Taiwan, Republic of China) from May 2 to May 13, 2003, and received mechanical ventilatory assistance from May 2 to May 10. A 0.023-μm filter was attached to the exhalation circuit.

Air sampling. After informed consent was obtained from the patient through interviews, air samples were collected from the isolated patient room from May 3 to May 13, 2003. The air sampler and filter cassette were placed approximately 1 m from the patient's bed. The sampling height was 1.2-1.5 m above the floor, approximately in the human breathing zone. The indoor air was filtered through a filter cassette with a 1-μm polytetrafluoroethylene (PTFE) filter at an airflow rate of 4.5 L/minute for 8 hours. After air sampling, filters were immediately stored at -70°C.

Aerosol generation. To evaluate the removal efficiency for airborne SARS-CoV of both the 0.023-μm and 0.3-μm HEPA filters connected to the breathing circuit, SARS-CoV virucidal sprays were generated using a small-volume nebulizer.[14] This nebulized solution consisted of diluted SARS-CoV and phosphate-buffered saline, and the median tissue culture infecting dose of SARS-CoV was 100. Various filters, including 0.2-μm PTFE filters and 0.2-μm polycarbonate (PC) filters, were used for collection of aerosolized SARS-CoV at a flow rate of 4.5 L/minute for 20 minutes.

Filter analysis. Prepared 1,120-μL aliquots of AVL buffer containing carrier RNA (Qiagen QIAamp Viral RNA Mini Kit) and 280 μL of phosphate-buffered saline were pipetted into a 60-mm Petri dish. These Petri dishes were placed on a shaker, and the filters were stripped for 20 minutes at room temperature. The stripping solution was pipetted into a 15-mL sterile microcentrifuge tube, and 1,120 μL of 99% alcohol was then added to stop all reactions. To extract the RNA from the filter samples, we used the Qiagen QIAamp Viral RNA Mini Kit and followed the manufacturer's protocol.

Real-time reverse-transcriptase PCR assay. After extraction, the viral RNA was quantitatively measured using the real-time reverse-transcriptase (RT) PCR method, according to the protocol specified by Taiwan's Center for Disease Control and Prevention.[15] The assay included transcription reaction at 48°C for 30 minutes; Taq polymerase activation at 95°C for 10 minutes; and PCR reaction, which consisted of denaturation at 95°C for 15 seconds and primer annealing extension at 60°C for 1 minute for 40 replication cycles.

RESULTS

Eight samples (2,160 L of air for a period of 8 hours in each sample) were obtained from the patient's room on May 3-10, 2003 (while the patient was receiving ventilatory assistance), and 3 were obtained on May 11-13, 2003 (after extubation); 1 sample was obtained in a negative-pressure room with a non-SARS patient, and 3 unexposed filters were tested.

TABLE.   Removal Efficiency of 2 Types of High-Efficiency Particulate Air (HEPA) Filters for Aerosolized Severe Acute Respiratory Syndrome Coronavirus

Sampling method	Proportion (%) of samples with positive PCR results, by type of filter	
	0.2-μm PTFE filter	0.2-μm PC filter
Aerosols passing through a 0.023-μm HEPA filter	0/3 (0)	0/3 (0)
Aerosols passing through a 0.3-μm HEPA filter	3/3 (100)	3/3 (100)

NOTE.   PC = polycarbonate; PCR = polymerase chain reaction; PTFE = polytetrafluoroethylene.

All samples exhibited negative SARS-CoV PCR results.

To evaluate the removal efficiency of the 0.023-μm HEPA filters connected to the breathing circuit, aerosolized SARS-CoV samples were generated and collected using 0.2-μm PTFE filters or 0.2-μm PC filters after passage through a 0.023-μm HEPA filter. Positive PCR results were obtained for none of these samples from the different sampling filters (PTFE filter and PC filter) (Table). However, although aerosols containing SARS-CoV do pass through a 0.3-μm HEPA filter, 100% of samples from both the 0.2-μm PTFE filters and 0.2-μm PC filters yielded positive PCR results. The control samples from the environment yielded negative PCR results for SARS-CoV DNA products.

DISCUSSION

Indoor environments in healthcare settings have various risk factors for infections, especially bioaerosol contamination. To date, few studies have addressed the characteristics of airborne SARS-CoV in healthcare settings.[14] Previous studies have collected aerosolized rhinovirus samples on PTFE filters with a 2-μm pore size at a rate of 8.5 L/minute for 10 minutes and analyzed with a seminested RT-PCR assay.[13] In our previous studies, airborne SARS-CoV samples were collected on a 1-μm PTFE filter at 4.5 L/minute for 8 hours and analyzed by real-time RT-PCR assay.[14] Also, we found that different filters (1-μm and 0.2-μm PTFE filters and a 0.2-μm PC filter) operated at flow rate of 4.5 L/min for 20 minutes yielded 100% positive results for SARS-CoV PCR.

We tried to collect airborne SARS-CoV samples using 1-μm PTFE filters in the our investigation. The results produced no positive PCR results in the air of the negative pressure isolation patient room with an intubated patient. A possible reason for this finding is the use of a 0.023-μm HEPA filter connected to the breathing circuit. The removal efficiency of HEPA filters with a 0.023-μm or 0.3-μm pore size was evaluated in this study. We found that HEPA filters with a pore size of 0.023 μm could remove 100% of aerosolized SARS-CoV. We demonstrated that HEPA filtration in the ventilator circuit might reduce the concentration of virus in ambient air surrounding an intubated patient to undetectable levels.

Moreover, the room air was not found to contain SARS-CoV DNA products after the patient was extubated. A possible explanation for this finding is the combined effect of clinical therapy for the patient and lower SARS-CoV concentrations in the room air. Because we studied only one patient receiving mechanical ventilatory assistance in our study, and we collected no samples without HEPA filtration in the ventilator circuit, we will consider attempting to reproduce our findings if more patients with SARS become available for study.

We sought to detect SARS-CoV in a negative-pressure isolation room housing a SARS patient receiving mechanical ventilatory support by analysis with a real-time RT-PCR amplification method. Our study found that SARS-CoV was detected by the real-time RT-PCR and that aerosols containing SARS-CoV did pass through a 0.3-μm HEPA filter. The sensitive real-time RT-PCR analytical method should be used to evaluate other types of bioaerosol contamination in healthcare settings to monitor the indoor air quality and protect the public and healthcare personnel in hospitals.

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