

Airborne SARS-CoV-2 and the Use of Masks for Protection against Its Spread in Wuhan, China

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Abstract:

The outbreak of COVID-19 has caused a global public health crisis. The spread of SARS-CoV-2 by contact is widely accepted, but the relative importance of aerosol transmission for the spread of COVID-19 is controversial. Here we characterize the distribution of SARS-CoV-2 in 123 aerosol samples, 63 masks, and 30 surface samples collected at various locations in Wuhan, China. The positive percentages of viral RNA included 21% of the aerosol samples from an intensive care unit and 39% of the masks from patients with a range of conditions. A viable virus was isolated from the surgical mask of one critically ill patient while all viral RNA positive aerosol samples were cultured negative. The SARS-CoV-2 detected in masks from patients, ambient air, and respirators from health workers compose a chain of emission, transport, and recipient of the virus. Our results indicate that masks are effective in protecting against the spread of viruses, and it is strongly recommended that people throughout the world wear masks to break the chain of virus transmission and thus protect themselves and others from SARS-CoV-2.

Keywords: SARS-CoV-2; Airborne; Mask

Text:

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an outbreak of COVID-19 (2019 coronavirus infectious disease) that has led to a global public health crisis (1-2). The spread of SARS-CoV-2 can be rapid (3), but there is still considerable controversy about its main route of transmission (4-5); that is, whether transmission is by contact or through the air (6-7). In particular, the importance of contact transmission has been widely recognized, but there is still considerable uncertainty regarding the relative importance of droplets versus aerosol particles (4) for the spread of the virus (<https://www.nature.com/articles/d41586-020-00974-w>). Previous studies have shown that these two airborne types of transmission are closely related to the spread of various respiratory pathogens (5, 8). Nonetheless,

it is still unclear whether the SARS-CoV-2 is spread by aerosols (4), and this has impeded the implement of control strategies. The purpose of this study was to evaluate the airborne transmission of SARS-CoV-2 through a field investigation of its occurrence in the air and other types of samples from health facilities in Wuhan, China.

Airborne distribution of SARS-CoV-2 in Wuhan

Exhaled air from both asymptomatic and symptomatic patients can be a source of airborne virus particles (5, 7). To characterize the distribution of the virus in selected microenvironments, 123 indoor and outdoor air samples were collected at various locations in the Jinyintan, Hongshan Square Cabin, and Union hospitals in Wuhan, China from 16 February 2020 to 14 March 2020. The qRT-PCR targeting to *Orf1ab* gene was used to quantify the genome copy numbers of the SARS-CoV-2. We found that 8 aerosol samples out of 38 (21.1%) from intensive care units (ICUs) and 1 aerosol sample out of 6 (16.7%) from Computerized Tomography (CT) rooms were positive for viral RNA (Figure 1). The range of virus concentrations in the positive aerosol samples was 1.11×10^3 to 1.12×10^4 RNA copies m^{-3} (Table 1). The relatively high virus RNA detection rates in the ICU ward and CT room where patients' activities were concentrated are consistent with a previous study (9). The aerosol samples from other areas of the hospitals, including medical staff rest areas and passageways were all negative, which may be an outcome of good ventilation and reflect clean air in those areas. For perspective, primary case transmission of COVID-19 in a closed environment was found to be 18.7 times greater compared to that from an open-air environment (10), and hospital settings are generally considered especially high-risk transmission areas. Indeed, SARS-CoV-2 infection of anesthesiologists after endotracheal intubation for confirmed COVID-19 patients have been reported in hospitals (11).

We found positive viral RNA in air samples collected 10-m away from an inpatient and outpatient building, with a concentration range of $0.65\text{--}8.92 \times 10^3$ RNA copies m^{-3} . Setti et al. (12) have similarly reported positive SARS-CoV-2 signals in

outdoor air samples from Bergamo, Northern Italy. No viral RNA was detected from any of the other areas sampled, including a residential community and an open public area; this indicates that the ambient air was generally not infectious and safe for the public.

To determine whether the virus in the air samples was viable, all of the viral RNA positive aerosol samples were subjected to cell culture for live virus isolation. The viral nucleic acid tests were negative after three passages of Vero-E6 cells inoculated in a blind test. Similarly, no viable virus was isolated from SARS-CoV-2 PCR-positive air samples in a study conducted at Nebraska, USA (7). Another study showed that SARS-CoV-2 can survive for ~3 h on aerosol particles (13). Thus, our experimental results support other findings which have shown that SARS-CoV-2 has a relatively short survival time in the aerosol, and this may be the reason that no viral replication in the air samples was detected.

Distribution of SARS-CoV-2 in masks and respirators

Air exhaled by patients infected with COVID-19 can spread SARS-CoV-2, especially those patients with high viral-load presentations (14). As a preventative measure, all patients in Wuhan hospitals were required to wear masks during hospitalization. To determine whether the surgical masks prevented the patients from exhaling virus, we collected 23 surgical masks from patients who had a range of health conditions, from critically to severely to mildly ill, and then tested the masks for the presence of virus (Figure 2). qRT-PCR results showed that 39.1% (9 of 23) of the masks were positive, and the positive detection rates ranged from 30% to 50% in the three types of patients (Fig. 2A). The numbers of viral RNA copies varied from 8.95×10^3 to 1.90×10^7 copies/mask (Fig. 2B).

All the positive masks were subjected to cell culture and inoculated with Vero-E6 cells after blind passage for three generations. One mask from a critically ill patient was detected positive, suggesting that the mask did indeed block the spread of viable virus in the air exhaled from the patient. Surgical masks have been shown to prevent transmission of human coronaviruses and influenza viruses from

symptomatic individuals, and there are strong indications that masks can reduce or prevent the transmission of viruses through air exhaled by patients (5). To evaluate the role of masks in preventing the spread of viruses to healthy people, we tested 10 filters from respirators and 40 masks of healthy researchers working in the P3 laboratory. The results showed that all the respirator filters were positive for SARS-CoV-2 RNA while the masks were all negative, indicating that the respirator filters worked effectively in blocking the transmission of virus in the air. The negative results for the masks, which were positioned downstream of the respirators, suggests that the respirators provide a high level of filtration efficiency for airborne virus particles and that the air inhaled by the researchers who wore them was not contaminated with virus.

Distribution of SARS-CoV-2 in samples collected near patients

Airborne virus can be deposited onto surfaces near patients, and high viral loads on surfaces around COVID-19 patients can reflect the distribution or spread of the virus through the air. SARS-CoV-2 RNA previously has been detected from surface samples from hospitals, including floors, window ledges, toilets, personal items, medical equipment, window benches, trashcans, sickbed handrails, etc. (7, 9, 16-20). Chia et al. (15) found that the presence and concentrations of SARS-CoV-2 in air and high-contact surface samples correlated with the occurrence of the illness and nasopharyngeal viral loads of COVID-19 patients. We collected environmental samples near COVID-19 patients in the hospitals, and of these, five surface swabs (cabinet, sick bedrail, door handle, and patient monitor) out of 24 (20.8%) from the ICU were positive for SARS-CoV-2, with viral RNA (Fig. 3A) ranging from 1.52×10^3 to 4.49×10^3 copies/swab (Fig. 3B).

The viruses on these surfaces presumably were from two sources: the first was when an infected patient's hand or other part of the body directly contacted a surface while the other resulted from virus in the patients' exhalations being deposited onto surfaces (20). Thus, airborne virus can increase the infection risk from indirect transmission through the deposition channel. In addition, our results imply that

efficient disinfection is critical for hospital infection control and protection of medical staff. Positive virus RNA was found for surface samples from a cabinet (2 of 2, 100%), sick patient's bedrail (1 of 2, 50%), door handle (1 of 2, 50%) and patient monitor (1 of 2, 50%). However, after rigorous disinfection, no viral RNA was detected in second batch samples from the same places.

Possible airborne transmission of the SARS-CoV-2

A comparison of contact versus airborne virus transmission and protective measures is presented in Figure 4A based on our findings and other research. Direct close contact was likely the most common method for transmission among medical staff at the beginning of the outbreak in January 2020 (18, 21). Later, indirect and airborne transmission probably became the more common way in which the virus spread as awareness of virus increased throughout the world and measures were taken to prevent contact transmission.

Based on the percentages of the virus-positive samples found in various microenvironments, we estimate the close-range infection risk by airborne droplet transmission may reach 30–50% (Figure 4B). The positive percentage of virus in the indoor environments with poor ventilation was ~20%, and this highlights the fact that ventilation is one of the key determinants of infection risk. In contrast, the virus-positive proportion for air samples from well-ventilated, non-hospital environments was zero (Table 1, Figure 4A). The SARS-CoV-2 positive masks from patients, ambient air, and respirators compose a chain from emission to transport to acquisition of the virus (Figure 4), and this is compelling evidence for the airborne transmission of SARS-CoV-2. It is noteworthy that a new type of air purifier with plasma and high filtration technology was deployed to control the virus in the air of a CT room at Jinyintan Hospital. The concentration of viral RNA decreased from 8.95×10^3 to undetectable levels after running the air purifier for 1 h, and this demonstrates the feasibility of removing the virus from indoor air. The airborne transmission and infection risk for the virus mainly occurs in closed environments where infected patients are present (Figure 4).

A recent mechanistic modeling study showed that short-range airborne transmission also dominates the exposure risks during close contact (22). Milton et al. (23) suggested that the infectious dose for virus in aerosols is about two orders of magnitude lower than that for large droplets, and they emphasized that fine aerosols play an important role in the transmission of seasonal influenza. Roy and Milton (24) have argued that SARS-CoV is not transmitted by either classical droplet or airborne transmission, but instead by a process somewhere between the two. These findings highlight the urgent need for detailed epidemiological information on the role of droplets and aerosols in the transmission of SARS-CoV-2, and even though the relative contribution of the airborne transmission route is difficult to quantify, methods to minimize transmission by this route should be included in plans to contain COVID-19.

Wearing masks prevents the spread of SARS-CoV-2

Wearing masks is the most basic protective measure to prevent patients from releasing the source virus into the air, and masks are often worn as personal protective equipment by healthy people (25). The use of surgical masks resulted in zero nosocomial transmissions of SARS-CoV-2 in a Hong Kong hospital (16), and that protective measure has been shown to reduce the copy numbers of influenza virus (23). Therefore, we conclude that masks are an effective, low-cost protective measure that can help prevent the airborne transmission of the virus, and wearing masks likely affords a significant level protection against COVID-19. Indeed, masks played a key role in the success of rapid control of the coronavirus epidemic in China especially in Wuhan. For example, the national mask production capacity in China was 20 million pieces/day in the early February, but it quickly expanded to 100 million pieces/day in less than one month (http://www.gov.cn/xinwen/2020-03/02/content_5485609.htm), and this helped mitigate the spread of the virus in China. At present, wearing masks is still mandated for on all occasions where people gather in China even though the pandemic has been largely controlled. The predilection for wearing masks is one of the most important differences in response to

the coronavirus pandemic among the United States, Europe, and China (<https://www.sciencemag.org/news/2020/03/not-wearing-masks-protect-against-coronavirus-big-mistake-top-chinese-scientist-says>). Our study demonstrates that masks are effective in limiting the spread of viruses in health care facilities, and more generally, we strongly recommend wearing masks to break the chain of transmission of SARS-CoV-2 and other respiratory viruses.

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Materials and Methods

Sampling

From February 16 to March 14, 2020, aerosol samples were collected over 30 min intervals with the use of a centrifugal aerosol-to-hydrosol sampler (WA-400, Beijing Dingblue Technology Co., Ltd., China) that operated at a flow rate of 400 L/min and had a 50% aerodynamic equivalent cut-off diameter of 0.8 μm . The aerosols were dissolved in 2 ml of phosphate buffer saline (PBS) containing antibiotic-antimycotic (Life Technologies Co., NY, USA) and 0.5% bovine serum albumin (BSA) (Amersco, Ohio, USA). A total of 81 aerosol samples were taken from selected locations in the Wuhan Jinyintan Hospital, Hongshan Square Mobile Hospital and Union Hospital, Tongji Medical College, and Huazhong University of Sciences and Technology. An additional 42 aerosol samples were collected in medical observation hotels, residential communities, gardens, and greenways. Twenty-three masks from patients and 24 swabs from surfaces in ICUs were also collected and analyzed. Ten 3M™ Versaflo™ TR-600 respirator filters (3M, Minnesota, USA) and 40 masks from healthy workers in the P3 lab of Wuhan Institute of Virology were collected for viral RNA detection.

RNA extraction

The swabs and patient masks were washed 6 times with 1ml PBS, and 1 ml of TRIzol™ Reagent (Invitrogen, NY, USA) was added to 1 ml of the aerosol washings. The viral RNA was isolated following a standard protocol according to the manufacturer's instructions and dissolved in 20 μl of Diethyl Pyrocarbonate (DEPC)-treated water.

qRT-PCR

qRT-PCR was performed using One Step PrimeScript™ RT-PCR kits (Perfect Real Time, Takara, RR064A), following the standard protocol as provided by the

manufacture. The *Orflab* primers (*Orflab*-F: 5'-CCC TGT GGG TTT TAC ACT TAA-3', *Orflab*-R: 5'-ACG ATT GTG CAT CAG CTG A-3') and probe (*Orflab*-P 5'-FAM-CCG TCT GCG GTA TGT GGA AAG GTT ATG G-BHQ1-3') were used. The *N* gene primers (*N*-F: 5'-GGG GAA CTTC TCC TGC TAG AAT-3', *N*-R: 5'-CAG ACA TTT TGC TCT CAA GCT G-3') and probe (*N*-P 5'-FAM-TTG CTG CTG CTT GAC AGA TT-TAMRA-3') were used to confirm the RNA samples with Cycle Threshold values between 37 and 40. Viral genome copy numbers were calculated based on a standard curve generated from the *in vitro* transcribed RNAs that contained the PCR amplicon. All the sequences and primes were designed referring to the SARS-CoV-2 genome (GISAID, <https://www.gisaid.org/>, accession number: EPI_ISL-402124)(1).

Isolation of SARS-CoV-2 from ICU aerosols and masks

Vero-E6 cells (ATCC CRL-1586) were cultured in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Thornton, Australia) and 100 units/mL penicillin and 1 mg/mL streptomycin (Invitrogen, NY, USA) in a humidified 37°C incubator with 5% CO₂. Three hundred µl of the collected solution from the air samples or patients' masks was directly inoculated into the cells. At 2 h post-inoculation, the cell media were refreshed. The inoculated cells were passaged for three rounds every 3 days. The culture media was collected for viral RNA quantification by qRT-PCR.

Author Contributions

Study design: Guan WX, Cao JJ; Sample collection: Lei CF, Hu J, Liu WH, Sun XL, Guan WX; Data analysis: Lei CF, Hu J, Guan WX, Deng F, Su ZY, Chen Z, Pei RJ; Data interpretation: Guan WX, Cao, JJ, Hu J, Deng F; Figures: Sun XL, Cao JJ; Writing: Guan WX, Cao JJ.

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We declare no competing interests.

ABBREVIATIONS

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, 2019 coronavirus infectious disease; WHO, World Health Organization; qRT-PCR, quantitative Reverse Transcription real-time fluorescence Polymerase Chain Reaction; ICU, intensive care units.

Figure legends

Figure 1. Detection of SARS-CoV-2 in the aerosol samples in Wuhan, China.

Aerosol samples from various locations were collected over 30 min with centrifugal bioaerosol samplers. qRT-PCR targeting the *Orflab* gene of the SARS-CoV-2 was performed using One Step PrimeScript™ RT-PCR kits.

A&C: The numbers of qRT-PCR positive and negative aerosol samples from indoor (A) or outdoor (C) are shown as filled and open bars, respectively; the dash line represents the qRT-PCR positive rates of the aerosol samples;

B&D: Copy numbers of SARS-CoV-2 RNA in aerosols from indoor (B) or outdoor (D). A standard curve ($Y = 47.951 - 3.44 \times \log(\text{copy numbers})$) generated from the *in vitro* transcribed RNA was used to calculate the copy numbers of SARS-CoV-2 RNA.

Figure 2. Detection of SARS-CoV-2 in the masks and filters of respirators in Wuhan, China

The masks that were collected from different patients and healthy staff in the P3 lab, and respirator filters were tested for SARS-CoV-2 RNA by qRT-PCR.

- A. The numbers of qRT-PCR positive and negative samples are shown as filled and open bars, respectively; the dash line represents the qRT-PCR positive rates for the aerosol samples;
- B. Copy numbers of SARS-CoV-2 RNA in the aerosols. The copy numbers were calculated as described in the legend of Figure. 1.

Figure 3. Detection of SARS-CoV-2 on solid surfaces in Wuhan hospitals.

Twenty four swabs from the solid surfaces in ICUs were collected and washed with PBS. The wash buffer was mixed with TRIzol Reagent to isolate RNA.

- A. The numbers of qRT-PCR positive and negative samples are shown as filled and open bars, respectively; the dash line represents the qRT-PCR positive rates for the solid surface samples;

B: Copy numbers of SARS-CoV-2 RNA on solid surfaces; these were calculated as described in the legend of Figure. 1.

Figure 4. Comparison of contact and airborne transmission of SARS-CoV-2

- A. Illustration showing a comparison of contact versus airborne transmission of SARS-CoV-2. The virus is shed from a COVID-19 infected individual with exhaled air, including droplets and aerosols. Contact transmission includes direct physical contact and indirect contact through various surfaces contaminated by the infected individual. Airborne transmission occurs through droplets exhaled from infected persons and aerosols that are produced through evaporation from droplets on timescales of milliseconds.
- B. Comparison of the characteristics and major control methods for contact and airborne transmission. Masks are recommended as the first line of personal protection equipment for both infected and susceptible/healthy individuals to minimize the spread of virus through airborne transmission.