COVID-19 patients in earlier stages exhaled millions of SARS-CoV-2 per hour

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Abstract

Exhaled breath samples had the highest positive rate (26.9%, n=52), followed by surface swabs (5.4%, n=242), and air samples (3.8%, n=26). COVID-19 patients recruited in Beijing exhaled millions of SARS-CoV-2 RNA copies into the air per hour. Exhaled breath emission may play an important role in the COVID-19 transmission.

Key words COVID-19; SARS-CoV-2; Exhaled breath; Airborne transmission

Introduction

The COVID-19 pandemic has left a major mark on human history. Global efforts to intervene the spread are accelerating. However, scientific information on the major routes of COVID-19 transmission is required. Analysis of environmental samples provides clues [1-4]. Notably, SARS-CoV-2 has been detected in air [2-4], on ventilation fans [1] and hospital floors [1,4]. Surface swabs from keyboards, cell phones, and patients' hands have also tested positive [1]. Other studies have shown that aerosolized SARS-CoV-2 not only survives on various surfaces for sustained periods of time [5], but also remains viable in the air for up to 3 hours [6]. Despite these rapid developments, the key COVID-19 transmission routes still remain debated [7], and evidence is extremely sparse on how SARS-CoV-2 is emitted into the air. Recently, scientists called for a recognition of airborne transmission of COVID-19 [8], and World Health Organization (WHO) made a change to the guideline accordingly, i.e., not excluding airborne transmission in crowed and closed settings. Here, we mainly investigated the breath emission of SARS-CoV-2 from 49 COVID-19 patients recruited in Beijing in addition to its environmental detection.

Methods

We recruited a total of 76 subjects, including 57 patients with COVID-19, four patients without COVID-19 from Hospital A and Hospital B, and 15 healthy subjects in Beijing (Table S1). Exhaled breath condensate (EBC) samples were collected from 20 imported COVID-19 patients from Canada, France, Iran, Italy, Japan, Spain, Thailand, United Kingdom, United States, and 29 local cases from Beijing (Table S2). Fig. S1 and Fig. S2 show the intensive care unit (ICU) and general ward floor settings of Hospital A, respectively. EBC samples were collected using a BioScreen device developed by Peking University (Fig S3). A total of 52 EBC

samples were collected from 49 COVID-19 patients (Table S2). EBC samples were also collected from 15 healthy subjects as controls. Twenty-six air samples were taken using two impingers (Fig S4; Table S3) as described in Supporting Information. A total of 242 surface swabs (10 or 25 cm^2) in quarantine hotels and hospitals or personal items from COVID-19 patients were obtained using wet cotton swabs (Table S4). All the samples collected were analyzed using RT-PCR (Roche 96 fluorescence qPCR instrument, Roche Molecular Systems, Inc., Pleasanton, CA) for SARS-CoV-2 targeting both ORF1ab and N genes using a detection kit (Jiangsu Bioperfectus Technologies, Nanjing, China). The quantitative estimates of viral loads in all samples were performed using the RNA amplification equation, and experimental and calculation details are described in Supporting Information. The ethics involving human subjects including the non-invasive collection of exhaled breath condensate samples was waived due to the urgency of the infectious disease outbreak, and approved by the Ethics Committee of the Center for Disease Control and Prevention of Chaoyang District of Beijing.

Results

The overall SARS-CoV-2 positive rate for EBC samples was 26.9% (n=52), while surface swabs and air samples had low positive rates of (5.4%, n=242), and (3.8%, n=26), respectively (Table 1). Cycle threshold (Ct) values (35.54±3.14) were obtained for each positive EBC sample (Table 1). The Ct values for EBC samples varied greatly among the patients, with lower values generally detected for earlier disease stages (Table S2). The breath emission rate was estimated to be from 1.03×10^5 to 2.25×10^7 viruses per hour (n=14) (Table1). The detection kit had different amplification efficiencies for ORF1ab and N target

genes of EBC samples (Table S2). Although EBC samples from two patients (A and B) were shown to contain SARS-CoV-2 (Table S2), surface swabs from their cell phones, hands, and toilet surfaces were negative for the virus (Table S4). In the ward of patient C, the virus was present on the surface of an air ventilation duct entrance that was located below the patient's bed (Video S2). In addition to causing air contamination, the exhaled SARS-CoV-2 could be partially responsible for the contamination on the surfaces that was observed.

From 26 air samples collected including those using a robot (Video S1), one sample (air-1) from an unventilated quarantine hotel toilet room was positive (estimated to be 6.07×10^3 viruses/m^3) (Table 1, Fig. S5, Table S4). Surface swab samples from a pillow case (Swab-1) and hands (Swab-2) of patient D who used the toilet room were shown to contain SARS-CoV-2, but no virus was detected in this patient's EBC sample which was collected on a different date (Fig. S5). Additionally, SARS-CoV-2 was detected on an air ventilation duct entrance surface as described above (the duct acted like an air sampler) (Fig S5). These air sample data, despite the low positive rate, still show that the air spaces of the hospitals housing the COVID-19 patients were contaminated with SARS-CoV-2.

Out of 242 surface swab samples, 13 were positive for SARS-CoV-2 (Table 1, Fig. S6 and Table S4). Among the five categories of surfaces, the Toilet pit had the highest SARS-CoV-2 positive rate (16.7%, n=12), followed by the Hospital floor (12.5%, n=16), the Other surfaces (7.4%, n=27), the Patient touching surfaces (4.0%, n=149), and the Medical touching surfaces (2.6%, n=38). Cycle threshold (Ct) values (36.38±1.92) were obtained for each positive surface swab sample (Table 1; Table S4). The surface-borne viral level was

estimated to be from 7.10 x 10^3 to 1.72 x 10^5 viruses/cm^2 (Table1). For toilet pit swab (Swab-3), the EBC-1 of its associated patient E also tested positive. For the Patient touching surfaces group (149 samples), we detected six positives from hands of patient D, a pillow case of patient D, mobile phones of patients F and G, and computer keyboards of patients G and H (Fig. S6; Table S4). Surprisingly, only 2 out of 22 surface swabs from the mobile phones of COVID-19 patients tested positive (Fig. S6; Table S4). None of the 26 surface swabs collected from handles of various objects appeared positive for the virus (Table S4). These observations seemly do not support the widely-held belief that direct transmission by contact with surfaces plays a major role in COVID-19 spread.

Discussion

For the first time, we here report that the SARS-CoV-2 is released directly into the air via breathing by COVID-19 patients. The detection limit for SARS-CoV-2 by the RT-PCR was reported to be approximately 100 RNA copies per µL [9]. Using the equation described in Supporting Information, the observed Ct values show that SARS-CoV-2 levels in exhaled breath could reach 10^5-10^7 copies/m^3 if an average breathing rate of 12 L/min is assumed. The SARS-CoV-2 breath emission rate is affected by many factors such as disease stage, patient activity, and possibly age. We found that the SARS-CoV-2 breath emission rate into the air was the highest, up to 10^5 viruses per min, during the earlier stages of COVID-19. This finding was in line with a previous report that the highest SARS-CoV-2 load in throat swabs was observed at the time of symptom onset [10]. Another significant discovery from this

work is that SARS-CoV-2 emission was not, however, continuous at the same rate, but was rather a sporadic event. For example, two EBC samples (EBC-1, EBC-2) collected from the same patient E, but on different dates, using the same method returned different test results (Table S2).

SARS-CoV-2 has previously been detected in fine particles in hospital air [4]. A peak of fluorescence biological particle at around 1 μm was also detected in exhaled breath from healthy subjects [11]. The SARS-CoV-2 negative air samples (Fig. S5) may be due to low SARS-CoV-2 emissions, virus inactivation by disinfectants, and rapid dilution or removal of SARS-CoV-2 by fresh air flow (2.5 m^3/min for general hospital wards, Video S2; 12 air exchanges per hour for ICU rooms). The SARS-CoV-2 presence in the toilet room air might be due to the exhaled virus or the virus aerosolization from the toilet. The spread of COVID-19 by asymptomatic patients has been also documented [12]. The asymptomatic disease carriers do not, generally, cough or sneeze to generate respiratory droplets; thus, the observed transmission of the disease has been difficult to explain by respiratory droplet transmission, but is rather logical for a fine aerosol route.

The dominant SARS-CoV-2 transmission routes need to be intervened in order to effectively stop the ongoing COVID-19 pandemic. Large respiratory droplets and direct contact transmissions are presently cited as major transmission routes for the COVID-19 by WHO. In contrast, we show that the surfaces of mobile phones (n=22) and various handles (n=35) frequently used by COVID-19 patients presented very low probabilities of SARS-CoV-2 presence (9.0% and 0%, respectively). Airborne transmission of SARS-CoV-2 has already played an important role in documented real-life COVID-19 spread in semi-enclosed

environments [7,13]; for example, cluster infection incidents in a choir in Washington State, USA [14], and a restaurant in Guangzhou, China [15]. Though we did not study infectivity or transmission probability and other virus releasing activities such as talking and singing, our study demonstrates that exhaled breath emission plays an important role in SARS-CoV-2 emission into the air, which could have contributed greatly to the observed airborne cluster infections and the ongoing pandemic. Accordingly, measures such as enhanced ventilation and the use of face masks are essential to minimize the risk of infection by airborne SARS-CoV-2.

Notes

Author contributions: M.Y., and J.M. contributed to the study design. J.M., M.Y., X.Q., Z.Z., H.W., L.S., and J.G., contributed to sample collection and experiments. J.M., X.Q., M.Y. contributed to patients' recruitment, and clinical management. M.Y., J.M, X.L., H.C., L.Z., L.M., S.A.G., P.B., and R.C.F. contributed to data analysis, data interpretation, figure preparation and literature search. M.Y. wrote the manuscript draft, and all authors revised the manuscript. All authors reviewed and approved the final version of the report.

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Table 1 Detection of SARS-CoV-2 and its positive rates from 52 EBC samples collected from 49 COVID-19 patients, 26 air samples, and 242 surface swabs. SARS-CV-2 emission rate or concentration level in air or on surface was estimated based on an assumed amplification efficiency of 75%; and a RT-PCR detection limit of 100 copies/μL[10]. Lower and upper bounds of virus emission rates or levels corresponded to upper and lower bounds of Ct values.

	Exhaled breath condensate （n=52）	**Air sample** （n =26）	**Surface swabs**（n=242）
Sample SARS-CoV-2 positive rate	14/52 （26.9%）	1/26 (3.8%)	13/242 (5.4%)
Cycle Threshold (Ct) range* （N or ORF1a/b）	35.54±3.14	38.40	36.38±1.92
Estimated SARS-CoV-2 emission rate/level	(1.03 x 10^5, 2.25x 10^7) viruses/hour	6.07 x 10^3 viruses/m^3	(7.10 x 10^3, 1.72 x 10^5) viruses/cm^2

* Lower Ct values were used among those of N or ORF1a/b genes for presentation and viral estimation.