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Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals

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Nature **582**, 557–560 (2020)

307k Accesses | **459** Citations | **3709** Altmetric | [Metrics](#)

Abstract

The ongoing outbreak of coronavirus disease 2019 (COVID-19) has spread rapidly on a global scale. Although it is clear that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is transmitted through human respiratory droplets and direct contact, the potential for aerosol transmission is poorly understood^{1,2,3}. Here we investigated the aerodynamic nature of SARS-CoV-2 by measuring viral RNA in aerosols in different areas of two Wuhan hospitals during the outbreak of COVID-19 in February and March 2020. **The concentration of SARS-CoV-2 RNA in aerosols that was detected in isolation wards and ventilated patient rooms was very low, but it was higher in the toilet areas used by the patients.** Levels of airborne SARS-CoV-2 RNA in the most public areas was undetectable, except in two areas that were prone to crowding; this increase was possibly due to individuals infected with SARS-CoV-2 in the crowd. We found that some medical staff areas initially had high concentrations of viral RNA with aerosol size distributions that showed peaks in the submicrometre and/or supermicrometre regions; however,

these levels were reduced to undetectable levels after implementation of rigorous sanitization procedures. Although we have not established the infectivity of the virus detected in these hospital areas, we propose that SARS-CoV-2 may have the potential to be transmitted through aerosols. Our results indicate that room ventilation, open space, sanitization of protective apparel, and **proper use and disinfection of toilet areas can effectively limit the concentration of SARS-CoV-2 RNA in aerosols**. Future work should explore the infectivity of aerosolized virus.

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Main

The ongoing outbreak of COVID-19, which has been reported in 206 countries and areas, has resulted in 857,641 confirmed cases and 42,006 deaths globally as of 2 April 2020. Owing to the increasing threat caused by COVID-19 to global health, the World Health Organization (WHO) has declared the COVID-19 outbreak a pandemic and global public health emergency. The causative pathogen of the COVID-19 outbreak has been identified as a highly infectious novel coronavirus that is referred to as SARS-CoV-2^{4,5,6}. Reported transmission pathways of SARS-CoV-2 in humans include the inhalation of virus-laden liquid droplets, close contact with infected individuals and contact with surfaces that are contaminated with SARS-CoV-2¹. Moreover, aerosol transmission has been suggested to be an additional, yet important pathway, on the basis of clinical observations in confined spaces^{2,3}. There are many respiratory diseases that are spread through airborne routes, such as tuberculosis, measles and chickenpox^{7,8}. A retrospective cohort study conducted after the SARS epidemic—which was caused by SARS-CoV—in Hong Kong in 2003 suggested that airborne spread may have had an important role in the transmission of SARS⁹. At present, little is known about the aerodynamic characteristics and transmission pathways of SARS-CoV-2 in aerosols; in part because of the difficulties in sampling virus-containing aerosols in real-world settings and challenges in their quantification at low concentrations.

We analysed the occurrence of airborne SARS-CoV-2 and its aerosol deposition at 30 sites in two designated hospitals and public areas in Wuhan, China, and then quantified the copy counts of

SARS-CoV-2 in aerosol samples using a robust droplet-digital-PCR-based detection method (ddPCR)¹⁰. The two hospitals are exclusively used for the treatments of patients with COVID-19 during the outbreak; however, each hospital has unique characteristics that serve different purposes. Renmin Hospital of Wuhan University (hereafter, Renmin Hospital) is representative of grade-A tertiary hospitals that have been designated for the treatment of patients with severe symptoms of COVID-19. By contrast, Wuchang Fangcang Field Hospital (hereafter, Fangcang Hospital) is representative of the makeshift field hospitals that were converted from indoor sports facilities or exhibition centres to quarantine and treat patients with mild symptoms. The sampling locations were classified into three categories according to their accessibility by different groups: (1) patient areas, where the patients with COVID-19 have a physical presence—these include the intensive care units, coronary care units and ward rooms inside Renmin Hospital, a toilet and staff workstations inside Fangcang Hospital; (2) medical staff areas, the workplaces in the two hospitals that are exclusively accessed by medical staff who had direct contact with the patients; and (3) public areas, venues that are open to the general public (Supplementary Table 1). Three types of aerosol samples were collected: (1) aerosol samples of total suspended particles with no upper size limit to quantify RNA concentrations of SARS-CoV-2 in aerosols; (2) aerodynamic size-segregated aerosol samples to determine the size distribution of airborne SARS-CoV-2 droplets; and (3) aerosol deposition samples to determine the deposition rate of airborne SARS-CoV-2.

The existence of SARS-CoV-2 in aerosol samples was determined through the quantification of its genetic material (RNA). The concentrations of airborne SARS-CoV-2 at the different sites are shown in Table 1. In general, very low or undetectable concentrations of airborne SARS-CoV-2 were found in most of the patient areas of Renmin Hospital, suggesting that the negatively pressurized isolation and high air exchange rate inside the intensive care units, coronary care units and ward room of Renmin Hospital are very effective in limiting the airborne transmission of SARS-CoV-2. The highest concentration in patient areas was observed inside a patient mobile toilet room at Fangcang Hospital (19 copies m⁻³), which is a temporary single toilet room of approximate 1 m² in area without ventilation. Airborne SARS-CoV-2 may come from either the patient's breath or the aerosolization of the virus-laden aerosol from the faeces or urine of a patient during use^{11,12}. Although the infectivity of the virus is not known in this study, the results

also relate to the findings of another study¹³, which found positive test results of wipe samples from room surfaces of toilets used by patients infected with SARS-CoV-2. In medical staff areas, the two sampling sites in Renmin Hospital had low concentrations of 6 copies m⁻³, whereas the sites in Fangcang Hospital generally had higher concentrations. In particular, the protective-apparel removal rooms (PPARs) in three different zones inside Fangcang Hospital are among the upper range of the concentrations of airborne SARS-CoV-2, ranging from 16 to 42 copies m⁻³ in the first round of sampling. In public areas outside the hospitals, we found that most of the sites had undetectable or very low concentrations of SARS-CoV-2 aerosols (below 3 copies m⁻³), except for one crowd-gathering site about 1 m from the entrance of a department store that customers frequently passed through and a site next to Renmin Hospital, through which the public including outpatients walked. Although both sites were outside buildings, it is possible that individuals infected with SARS-CoV-2 in the crowd may have been the source of virus-laden aerosols during the sampling period. The results suggest that, overall, the risks of infection are low in well-ventilated or open public venues, but do reinforce the importance of avoiding crowded gatherings and implementing the early identification and diagnosis of individuals infected with SARS-CoV-2 for quarantine or treatment.

Table 1 RNA concentration of airborne SARS-CoV-2 at different locations in Wuhan

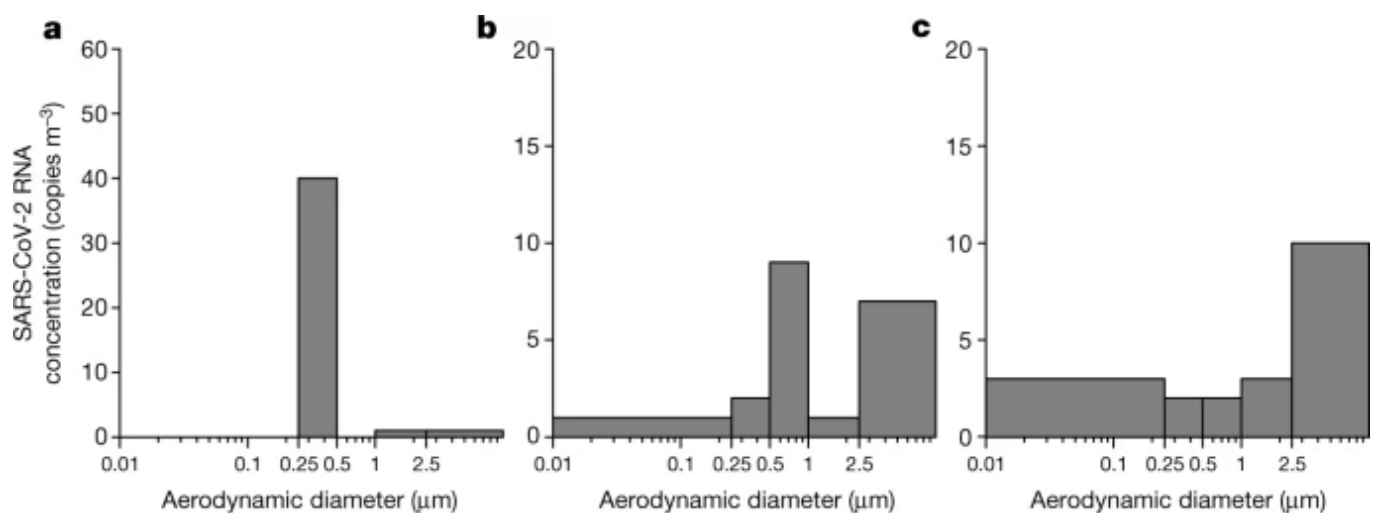
Inside a room of the intensive care unit of Renmin Hospital, the two aerosol deposition samples tested positive with an estimated deposition rate of 31 and 113 copies m⁻² h⁻¹, although the concentration of the total suspended particles in the aerosol sample inside this room of the intensive care unit was below the detection limit (Table 1). The sample with the higher deposition rate was placed in the hindrance-free corner of the room, approximately 3 m from the bed of a patient. The other sample, for which a lower number of virus copies was recorded, was placed in another corner, approximately 2 m from the bed of the patient and below medical equipment, which may have blocked the path of virus aerosols during sedimentation. Our findings, although based on a small sample size, indicate that virus-laden aerosol deposition may have a role in surface contamination and subsequent contact by susceptible people, which results in the infection of individuals with SARS-CoV-2.

In general, medical staff areas had higher concentrations of SARS-CoV-2 aerosols compared with patient areas in both hospitals during the first round of sampling (17–24 February 2020) at the peak of the COVID-19 outbreak (Table 1). For sampling sites at Renmin Hospital, the air circulation in medical staff areas is isolated by design from the air circulation in the patient rooms. By contrast, in Fangcang Hospital, the non-ventilated temporary PPAR was isolated from the patient hall, in which the aerosol concentration of SARS-CoV-2 was generally low. The second round of sampling of total suspended particles in medical staff areas of Fangcang Hospital was conducted after the number of patients reduced from more than 200 to less than 100 per zone and the implementation of more rigorous and thorough sanitization measures, including more frequent spraying of chlorinated disinfectant on the floor of patient areas, additional disinfection using 3% hydrogen peroxide in the PPAR at least once a week, thoroughly spraying alcohol disinfectant on the protective apparel before taking it off and an increased operation time of indoor air purifiers. The samples from this second round showed all undetectable results (Table 1), confirming the importance of sanitization in reducing the amount of airborne SARS-CoV-2 in high-risk areas.

SARS-CoV-2 aerosols were mainly found to include two size ranges, one in the submicrometre region (d_p between 0.25 and 1.0 μm) and the other in supermicrometre region ($d_p > 2.5 \mu\text{m}$). Aerosols in the submicrometre region were predominantly found in PPARs in zones B and C of Fangcang Hospital (Fig. 1a, b) with peak concentrations of 40 and 9 copies m^{-3} in the 0.25–0.5 μm and 0.5–1.0 μm range, respectively. By contrast, aerosols in the supermicrometre region were mainly observed in the PPAR of zone C of Fangcang Hospital (Fig. 1b) with concentrations of 7 copies m^{-3} . The medical staff's office (Fig. 1c) had more virus-laden aerosols in the supermicrometre size range, but the size distribution is flatter compared with the range in other areas. Reports on the resuspension of microorganisms from the floor, clothing and furniture have previously been noted to contribute to the generation of microbial aerosols in the built environment¹⁴. Therefore, we hypothesize that the source of the submicrometre peak is the resuspension of virus-laden aerosols from the surface of the protective apparel worn by medical staff while they are removing the equipment. The submicrometre virus-laden aerosols may originally come from the direct deposition of respiratory droplets or airborne SARS-CoV-2 from a patient onto the protective apparel as evidenced by the deposition samples (Table 1). The higher

mobility owing to their smaller aerodynamic diameter facilitates the resuspension from the surface of protective apparel after gaining the initial velocity while the equipment is being removed. On the other hand, floor-deposited SARS-CoV-2 is possibly the source of supermicrometre virus-laden aerosols and was carried across different areas by medical staff. Furthermore, a recent study has experimentally demonstrated that SARS-CoV-2 could maintain its biological stability in aerosols and on different surfaces for hours to days¹⁵. The submicrometre SARS-CoV-2 aerosols found in this study had a relatively longer residence time, indicating that the virus was probably still infectious during transmission.

Fig. 1: Concentration of airborne SARS-CoV-2 RNA in different aerosol size bins.



a, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone B of Fangcang Hospital. **b**, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone C of Fangcang Hospital. **c**, Concentration of SARS-CoV-2 in the medical staff's office of Fangcang Hospital. The x axis represents the aerodynamic diameter on a logarithmic scale to cover the multiple magnitudes of measured aerosol diameters.

[Source Data](#)

This study has its inherent limitations because of the small sample size and the description of sample viral RNA instead of virus infectivity, which was imposed by restricted access to the

patient and medical staff areas at the epicentre of the COVID-19 outbreak. Nonetheless, the findings of this study provide a real-world investigation of the aerodynamic characteristics of airborne SARS-CoV-2 in Wuhan, where a strict quarantine and travel restrictions were implemented during the peak of the COVID-19 outbreak. The findings suggest that toilet use by patients with COVID-19 and crowd gatherings that included individuals infected by SARS-CoV-2 are non-negligible sources of airborne SARS-CoV-2, although the infectivity of the virus is not known. We also describe a transmission pathway for SARS-CoV-2 aerosols that is mediated by the surface deposition of the virus on and resuspension from protective apparel of medical staff and the floor surface. The results of this study have important implications for the prevention of infection of the public and protection of medical staff. We call for particular attention to (1) the ventilation and sterilization of toilets as a potential source for the spreading of the virus; (2) personal protection measures for the general public, such as the wearing of masks and avoidance of busy crowds to reduce the risk of exposure to airborne virus; (3) the effective sanitization of high-risk areas in the hospital to limit the transmission of airborne SARS-CoV-2 and to protect the medical staff; (4) the effectiveness of a naturally ventilated large stadium to limit the aerosol transmission of SARS-CoV-2 when converted to a field hospital for the quarantine and treatment of patients with SARS-CoV-2; and (5) surface sanitization of the apparel before the equipment is taken off to help to reduce the potential risk of infection for medical staff.

Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Sample collection

The sampling was conducted between 17 February and 2 March 2020 in the locations in two rounds as shown in Table 1. All aerosol samples were collected on presterilized gelatin filters

(Sartorius). A total of 30 aerosol samples of total suspended particles were collected on 25-mm-diameter filters loaded into styrene filter cassettes (SKC) by sampling air at a fixed flow rate of 5.0 l min^{-1} using a portable pump (APEX2, Casella). A total of three size-segregated aerosol samples was collected using a miniature cascade impactor (Sioutas Impactor, SKC) that separated aerosols into five ranges ($>2.5 \text{ }\mu\text{m}$, $1.0\text{--}2.5 \text{ }\mu\text{m}$, $0.50\text{--}1.0 \text{ }\mu\text{m}$ and $0.25\text{--}0.50 \text{ }\mu\text{m}$ on 25-mm filter substrates, and $0\text{--}0.25 \text{ }\mu\text{m}$ on 37-mm filters) at a flow rate of 9.0 l min^{-1} . A total of two aerosol deposition samples was collected using 80-mm-diameter filters packed into a holder with an effective deposition area of 43.0 cm^2 and the filters were placed intact on the floor in two corners of the intensive care unit room of Renmin Hospital for 7 days. Sampling durations and operation periods are described in Supplementary Table 1. All sampling instruments were located in the centre of the respective sampling area, where the sampling inlet was at a height of 1.5 m from the floor. Considering the limited experimental conditions and the small sample size, the integrity and robustness of the experiment protocol was examined extensively in the laboratory before field sampling and these results are described in Supplementary Table 2.

Analytical method and data analysis

After the collection of aerosol samples, all samples were processed immediately in the BSL-2 laboratory of Wuhan University. The 25-, 37-mm and 80-mm filter samples were dissolved in deionized water, after which TRIzol LS reagent (Invitrogen) was added to inactivate SARS-CoV-2 viruses and extract RNA according to the manufacturer's instructions. First-strand cDNA was synthesized using the PrimeScript RT kit (TakaRa). Optimized ddPCR was used to detect the presence of SARS-CoV-2 viruses according to a previous study¹⁰. Analysis of the ddPCR data was performed using QuantaSoft software (Bio-Rad). The concentration reported by the procedure equals the number of copies of template per microlitre of the final $1\times$ ddPCR reaction, which was normalized to copies m^{-3} in all of the results; therefore, the virus or viral RNA concentration in aerosol is expressed in copies m^{-3} throughout. A detailed protocol is provided in the [Supplementary Information](#).

Reporting summary

Further information on research design is available in the [Nature Research Reporting Summary](#) linked to this paper.

Data availability

All data generated and analysed during this study are included in the Article and its [Supplementary Information](#). Source Data for Fig. 1 are provided with the paper.

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Acknowledgements

This study was supported by the Special Fund for COVID-19 Research of Wuhan University. We thank Taikang Insurance Group, Beijing Taikang Yicai Foundation, Renmin Hospital and Wuchang Fangcang Hospital for their support of this work; H. Xu from Xi'an Jiaotong University, Qingdao Laoying Environmental Technology, Beijing Top Science, Shanghai Leon Scientific Instrument, Shanghai Eureka Environmental Protection Hi-tech and Sapiens Environmental Technology for their support in providing the sampling devices and technical support of this study; and C. Wang, Q. Zhang, G. Liang and Z. Song for their assistance in filter sample preparation and logistics support.

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Contributions

K.L., Y.C., Z.N., Q.F., H.K. and K.-f.H. conceptualized the study design; Yuan Liu, Y.C., M.G., Yingle Liu and K.L. collected samples; Yuan Liu, M.G. and X.L. carried out the laboratory tests; Yuan Liu, Z.N., Y.C., N.K.G., M.G., X.L. and K.L. analysed the data; Y.C., Z.N., Yuan Liu, Q.F., H.K., J.C., K.-f.H. and K.L. interpreted the results; Yuan Liu and Z.N. wrote the initial drafts of the manuscript; Yuan Liu, Z.N., Y.C. and K.L. revised the manuscript; M.G., Yingle Liu, N.K.G., L.S., Y.D., J.C., D.W., K.X., H.K. and Q.F. commented on the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature* thanks Linsey Marr and the other anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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Supplementary information

Supplementary Information

This file contains Supplementary Methods, Supplementary Table 1 (Specifications of sampling sites) and Supplementary Table 2 (Cycle threshold value (Ct) of EV71 virus positive control and laboratory test air samples RT-PCR).

Reporting Summary Peer Review File

Source data

Source Data Fig. 1

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Cite this article

Liu, Y., Ning, Z., Chen, Y. *et al.* Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* **582**, 557–560 (2020). <https://doi.org/10.1038/s41586-020-2271-3>

Received 14 March 2020 **Accepted** 20 April 2020 **Published** 27 April 2020

Issue Date 25 June 2020 **DOI** <https://doi.org/10.1038/s41586-020-2271-3>

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