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

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Yuan Liu^{1,6}, Zhi Ning^{2,6,6}, Yu Chen^{1,6,6,7}, Ming Guo^{1,6}, Yingle Liu¹, Nirmal Kumar Gali², Li Sun², Yusen Duan³, Jing Cai⁴, Dane Westerdahl², Xinjin Liu¹, Ke Xu¹, Kin-fai Ho^{5⊠}, Haidong Kan^{4⊠}, Qinqyan Fu^{3 ⋈} & Ke Lan^{1 ⋈}

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 understood¹⁻³. 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.

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-2⁴⁻⁶. 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

State Key Laboratory of Virology, Modern Virology Research Center, College of Life Sciences, Wuhan University, Wuhan, P. R. China. 2Division of Environment and Sustainability, The Hong Kong University of Science and Technology, Hong Kong, P. R. China. ³Shanghai Environmental Monitoring Center, Shanghai, P. R. China. ⁴School of Public Health, Key Laboratory of Public Health Safety of the Ministry of Education and Key Laboratory of Health Technology Assessment of the Ministry of Health, Fudan University, Shanghai, P. R. China. 5JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, P. R. China. These authors contributed equally: Yuan Liu, Zhi Ning, Yu Chen, Ming Guo. Entail: zhining@ust.hk; chenyu@whu.edu.cn; kfho@cuhk.edu.hk; kanh@fudan.edu.cn; gingyanf@sheemc.cn; klan@whu.edu.cn

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

Category	Sites	Sample type	Concentration (copies m ⁻³)
Patient areas			
Fangcang Hospital	Zone A workstation ^a	TSP ^b	1
		TSP ^c	9
	Zone B workstation	TSP	1
	Zone C workstation ^a	TSP ^b	5
		TSP°	0
	Patient mobile toilet room	TSP	19
Renmin Hospital	Intensive care unit	TSP	0
	Intensive care unit	Deposition	31 ^d
	Intensive care unit	Deposition	113 ^d
	Coronary care unit	TSP	0
	Ward zone 16	TSP	0
Medical staff areas			
Fangcang Hospital	PPAR of zone A ^a	TSPb	16
		TSP°	0
	PPAR of zone B	Size-segregated	42
	PPAR of zone C ^a	Size-segregated ^b	20
		TSP°	0
	Male staff change room	TSP	20
	Female staff change room	TSP	11
	Medical staff's office	Size-segregated	20
	Meeting room	TSP	18
		TSP ^b	21
		TSP°	0
Renmin Hospital	Passageway for medical staff	TSP	6
	Dining room for medical staff	TSP	6
Public areas			
	Fangcang Hospital pharmacy	TSP	3
	Renmin Hospital doctor office	TSP	0
	Renmin Hospital outpatient hall	TSP	0
	Renmin Hospital outdoor	TSP	7
	University office doorside	TSP	0
	University hospital outpatient hall	TSP	0
	Community checkpoint	TSP	0
	Residential building	TSP	0
	Supermarket	TSP	0
	Department store 1	TSP	11
	Department store 2	TSP	3
	Blank control ^a	Field blank ^b	0
		Field blank ^c	0

TSP, total suspended particles. The samples were distinct by design owing to the unique conditions inside the hospitals during COVID-19 outbreak. We collected 35 samples (not including two blanks) at different sites, therefore n = 35. The replicability is limited by very restricted experimental conditions to conduct sampling in the highly infectious zones.

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).

^{*}Two rounds of sampling were conducted for the sites. A blank control was included for each of round of sampling. Detailed information is shown in Supplementary Table 1.

 $^{^{\}mathrm{b}}$ The samples were taken during the first round of sampling from 17 February to 24 February 2020.

The samples were taken during the second round of sampling on 2 March 2020.

 $^{^{\}rm d}$ The reported values are virus aerosol deposition rates in copies m $^{\rm -2}$ h $^{\rm -1}$.

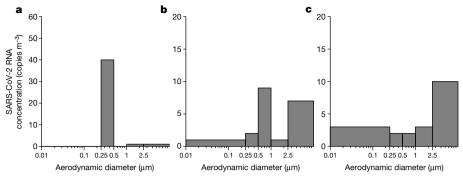


Fig. 1 | Concentration of airborne SARS-CoV-2 RNA in different aerosol size bins. a, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone B of Fangcang Hospital. b, Concentration of SARS-CoV-2 in a protective-apparel removal room in zone C of Fangcang Hospital.

c, Concentration of SARS-CoV-2 in the medical staff's office of Fangcang $Hospital. \ The \it x \, axis \, represents \, the \, aerodynamic \, diameter \, on \, a \, logarithmic$ scale to cover the multiple magnitudes of measured aerosol diameters.

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.

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 μ m) 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 μ m and 0.5–1.0 μ m 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

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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.

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 sta $dium \, to \, limit \, the \, aerosol \, transmission \, of \, SARS\text{-}CoV\text{-}2 \, when \, converted$ 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.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2271-3.

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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 µm, 1.0-2.5 µm, 0.50- $1.0 \,\mu\text{m}$ and $0.25-0.50 \,\mu\text{m}$ on 25-mm filter substrates, and $0-0.25 \,\mu\text{m}$ 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 cm² 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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Author 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.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-020-2271-3.

Correspondence and requests for materials should be addressed to Z.N., Y.C., K.-f.H., H.K., Q.F. or K.L.

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Reporting Summary

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Statistics	
For all statistical analys	es, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a Confirmed	
☐ ☐ The exact sam	ple size (n) for each experimental group/condition, given as a discrete number and unit of measurement
A statement of	n whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
X	test(s) used AND whether they are one- or two-sided ests should be described solely by name; describe more complex techniques in the Methods section.
A description	of all covariates tested
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	ion of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	hesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted exact values whenever suitable.
For Bayesian a	analysis, information on the choice of priors and Markov chain Monte Carlo settings
For hierarchic	al and complex designs, identification of the appropriate level for tests and full reporting of outcomes
Estimates of e	ffect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
·	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Software and c	ode
Policy information abou	ut <u>availability of computer code</u>
Data collection	NONE
Data analysis	QuantaSoft analysis software v.1.7.4.0917 (Bio-Rad) was used for analysis of the ddPCR data.
	m algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data	
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All data generated and ar containing Source Data for	nalysed during this study are included in the Article and its Supplementary Information files, which include an additional Excel file or Fig. 1 and all raw data.
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Life sciences	Behavioural & social sciences

Ecological, evolutionary & environmental sciences study design

All studies must disclose or	these points even when the disclosure is negative.			
Study description	his study investigated the aerodynamic nature and aerosol transmission of SARS-CoV-2 aerosol in Wuhan under strict quarantine and travel restriction during the peak of COVID-19 outbreak.			
Research sample	otal of 35 distinct samples of three types, total suspended particle, size segregated and aerosol deposition were collected.			
Sampling strategy	Ve sampled three types of virus aerosol samples at 30 sites covering patient and medical staff areas inside hospitals and in public reas in Wuhan. The sampling was designed to identify the hotspots of airborne SARS-CoV-2 and investigate their sources, and seek or evidences of their aerosol transmission across different isolation zones and air-surface transfer mechanisms.			
Data collection	e collected two batches of samples from Feb 17 to Feb 24, 2020 and on Mar 2, 2020 respectively.			
Timing and spatial scale	mpling durations range from 5 to 20 hours for total suspended particle and size segregated samples and 7 days for aerosol position samples. Sampling air volumes range from 1.5 m3 to 8.9 m3.			
Data exclusions	o data were excluded in this work.			
Reproducibility	e samples were distinct by design in this study due to the unique conditions inside the hospitals during COVID-19 outbreak.			
Randomization	This study categorized sampling locations by functions and user groups. Each sample has served different purposes so no randomization was attempted.			
Blinding	The sampling process itself has no impact on the study subject and data integrity by the nature of study, so no blinding was attempted.			
Did the study involve field work? Yes No Field work, collection and transport				
Field conditions	The sampling sites include indoor of hospital function areas and outdoor in public areas in Wuhan under strict quarantine and travel restriction.			
Location	We sampled SARS-CoV-2 aerosol samples at 30 sites in two designated hospitals and public areas in Wuhan.			
Access and import/expor	This field study didn't involve any study objects that require permission so no approval is needed.			
Disturbance	The sampling process has no disturbance of the subjects.			
Reporting for specific materials, systems and methods				
Ve require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ystem or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.				
Materials & experime	ntal systems Methods			
n/a Involved in the study	n/a Involved in the study			
Antibodies	ChIP-seq			
Eukaryotic cell lines Flow cytometry				
Palaeontology MRI-based neuroimaging				
Animals and other organisms Human research participants				
Clinical data				
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