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

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The ongoing COVID-19 outbreak has spread rapidly on a global scale. While the transmission of SARS-CoV-2 via human respiratory droplets and direct contact is clear, the potential for aerosol transmission is poorly understood^{1–3}. This study investigated the aerodynamic nature of SARS-CoV-2 by measuring viral RNA in aerosols in different areas of two Wuhan hospitals during the COVID-19 outbreak in February and March 2020. The concentration of SARS-CoV-2 RNA in aerosols detected in isolation wards and ventilated patient rooms was very low, but it was elevated in the patients' toilet areas. Levels of airborne SARS-CoV-2 RNA in the majority of public areas was undetectable except in two areas prone to crowding, possibly due to infected carriers in the crowd. We found that some medical staff areas initially had high concentrations of viral RNA with aerosol size distributions showing peaks in submicrometre and/or supermicrometre regions, but 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 via 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.

Circulating in 206 countries and areas, the COVID-19 outbreak has resulted in 857,641 confirmed cases including with 42,006 deaths globally as of April 2, 2020. Due to its increasing threat to global health, WHO has declared the COVID-19 outbreak as a pandemic and global public health emergency. The causative pathogen of the COVID-19 outbreak has been identified as a highly infectious novel coronavirus which is referred to as the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)^{4–6}. Reported transmission pathways of SARS-CoV-2 in humans are: 1) inhalation of virus laden liquid droplets 2) close contact with infected persons and 3) contact with surfaces contaminated with SARS-CoV-2¹. Moreover, aerosol transmission has been suggested as an additional yet important pathway from clinic observations in confined spaces^{2,3}. There are many respiratory diseases spread by the airborne route such as tuberculosis, measles and chickenpox^{7,8}. A retrospective cohort study conducted after the SARS epidemic in Hong Kong in 2003 suggested that airborne spread may have played an important role in the transmission of that disease⁹. At present, little is known on the aerodynamic characteristics and transmission pathways of SARS-CoV-2 in aerosols, partly due to the difficulties in sampling virus containing aerosol in real-world and challenges in their quantification at low concentration.

In this study, we sampled airborne SARS-CoV-2 and its aerosol deposition at 30 sites in two designated hospitals and public areas in Wuhan and then quantified the SARS-CoV-2 copy counts of aerosol samples using a robust droplet digital PCR-based detection method (ddPCR)¹⁰. The two hospitals are exclusively used for COVID-19 patient treatment during the outbreak but each with unique characteristics serving different purposes. The Renmin Hospital of Wuhan University (Renmin Hospital hereafter) represents Grade-A tertiary hospitals designated for treatment of severe symptom COVID-19 patients, while the Wuchang Fangcang Field Hospital (Fangcang Hospital hereafter) is representative of the make-shift field hospitals which was renovated 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 (PAA), where the COVID-19 patients have physical presence. These include the Intensive Care Units (ICU), Coronary Care Units (CCU) and ward rooms inside Renmin Hospital, a toilet and staff workstations inside Fangcang Hospital; 2) Medical Staff Areas (MSA), the workplaces in the two hospitals exclusively accessed by the medical staff who had direct contact with the patients, and 3) Public Areas (PUA), venues open for the general public (Supplementary Table 1). Three types of

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aerosol samples were collected: 1) Aerosol samples of total suspended particles (TSP) with no upper size limit to quantify RNA concentrations of SARS-CoV-2 in aerosol; 2) Aerodynamic size-segregated aerosol samples to determine the size distribution of airborne SARS-CoV-2; 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 airborne SARS-CoV-2 concentrations in different categorized sites are shown in Table 1. Generally very low or non-detectable concentrations of airborne SARS-CoV-2 were found in most PAA of Renmin Hospital, suggesting the negatively pressurized isolation and high air exchange rate inside ICU, CCU and ward room of Renmin Hospital are very effective in limiting the airborne transmission of SARS-CoV-2. The highest concentration in PAA was observed inside the Fangcang Hospital patient mobile toilet room (19 copies m^{-3}), which is a temporary single toilet room of approximate 1 m^2 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 patient's faeces or urine during use^{11,12}. Although the infectivity of the virus is not known in this study, the results also relate to the findings by Ong et al. that showed positive test results of wipe samples from room surfaces of toilets used by SARS-CoV-2 patients¹³. In MSAs, the two sampling sites in Renmin Hospital had low concentration of 6 copies m^{-3} , while the sites in Fangcang Hospital in general had higher concentrations. Particularly, the Protective Apparel Removal Rooms (PARRs) in three different zones inside Fangcang Hospital are among the upper range of airborne SARS-CoV-2 concentration from 16 to 42 copies m^{-3} in the first batch of sampling. In PUA outside the hospitals, we found the majority of the sites have undetectable or very low concentrations of SARS-CoV-2 aerosol (below 3 copies m^{-3}), except for one crowd gathering site about 1 m to the entrance of a department store with customers frequently passing through, and the other site next to Renmin Hospital where the public including outpatients passed by. While both sites were outside the building, it is possible that infected carriers of SARS-CoV-2 in the crowd may have contributed as the source of virus-laden aerosol during the sampling period. The results suggest overall low risks in the well ventilated or open public venues but do reinforce the importance of avoiding crowded gatherings and implementing early identification and diagnosis of infected carriers for quarantine or treatment.

Inside the Renmin Hospital ICU rooms, the two aerosol deposition samples tested positive with an estimated deposition rate of 31 and 113 copies $m^{-2} hr^{-1}$, although the TSP aerosol sample concentration inside this ICU room was below 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 patient's bed. The other sample recorded lower virus copies and it was placed in another corner, approximately 2 m from the patient's bed and below medical equipment which may have blocked the path of virus aerosol sediments. Our findings, though based on a small sample size, indicate virus-laden aerosol deposition may play a role in surface contamination and subsequent contact by susceptible people resulting in human infection.

MSAs in general had higher concentrations of SARS-CoV-2 aerosol compared to PAAs in both hospitals in the first batch of sampling during the peak of COVID-19 outbreak (Table 1). For Renmin Hospital sampling sites, the air circulation in MSA by design is isolated from that of the patient rooms. While for Fangcang Hospital, the non-ventilated temporary PARR was isolated from the patient hall where the SARS-CoV-2 aerosol concentration was generally low. The second batch of TSP sampling in Fangcang Hospital MSAs was conducted after number of patients reduced from over 200 to less than 100 per zone and implementation of more rigorous and thorough sanitization measures including more frequent spraying of chlorinated disinfectant on the floor of patient areas, additional disinfection by 3% hydrogen peroxide in the

PARR at least once a week, spraying alcohol disinfectant all over the protective apparel before taking off, and prolonged operation time of indoor air purifiers. The samples from this second batch showed all non-detectable results (Table 1), confirming the importance of sanitization in reducing the airborne SARS-CoV-2 in high risk areas.

SARS-CoV-2 aerosol mainly resides in two size ranges, one in the sub-micron region (d_p between 0.25 to 1.0 μm) and the other in supermicron region ($d_p > 2.5 \mu m$). The submicron region was dominantly noted in PARRs in Zone B and C of Fangcang Hospital (Fig. 1a and 1b) with peak concentration of 40 and 9 copies m^{-3} in 0.25 to 0.5 μm and 0.5 to 1.0 μm , respectively. Whereas the supermicron region was observed in Fangcang Hospital Zone C PARR (Fig. 1b) with 7 copies m^{-3} . The medical staff's office (Fig. 1c) had more virus-laden aerosol in the supermicron size range, but the size distribution is flatter compared with others. Reports on the resuspension of microorganisms from the floor, clothing, and furniture was noted to contribute to the generation of microbial aerosols in the built environment¹⁴. Therefore, we hypothesize the source of the submicron peak is the resuspension of virus-laden aerosol from the surface of medical staff protective apparel while they are being removed. The submicron virus-laden aerosol may originally come from the direct deposition of patient's respiratory droplets or airborne SARS-CoV-2 onto the protective apparel as evidenced by the deposition samples (Table 1). The higher mobility due to their smaller aerodynamic diameter facilitates the resuspension from apparel surface after gaining the initial velocity while being removed. On the other hand, the floor deposited SARS-CoV-2 is possibly the source of supermicron virus-laden aerosol and was carried across different areas by the medical staff. Furthermore, a recent study has experimentally demonstrated SARS-CoV-2 could maintain its biological stability in aerosol and on different surfaces for hours to days¹⁵. The submicron SARS-CoV-2 aerosol found in this study has relatively longer residence time implying they are probably still infectious during transmission.

This study had its inherent limitations in small sample size and representation of sample viral RNA instead of virus infectivity, imposed by restricted access to the patient and medical staff areas in the epicentre of the COVID-19 outbreak. Nonetheless, the findings from this study provide the first real-world investigation on the aerodynamic characteristics of airborne SARS-CoV-2 in Wuhan implemented with strict quarantine and travel restrictions during the peak of COVID-19 outbreak. The findings suggested the toilet use by and crowd gathering with COVID-19 infected individuals are non-negligible sources of airborne SARS-CoV-2 although its infectivity is not known. We also proposed a SARS-CoV-2 aerosol transmission pathway that is mediated by the surface deposition on and resuspension from medical staff protective apparel and surface floor. The results from this study have important implications for the public health prevention and medical staff protection. We call for particular attentions on 1) the ventilation and sterilization of toilets as a potential spread source of the virus; 2) personal protection measures for the general public such as wearing masks and avoiding busy crowd to reduce the risk of airborne virus exposure; 3) the effective sanitization of the high risk area in the hospital to limit the transmission of airborne SARS-CoV-2 and protect the medical staff; 4) the effectiveness of naturally ventilated large stadium to limit the aerosol transmission of SARS-CoV-2 when renovated to field hospital for patient quarantine and treatment; 5) surface sanitization of the apparel before they are taken off to help reduce the potential infection risk of 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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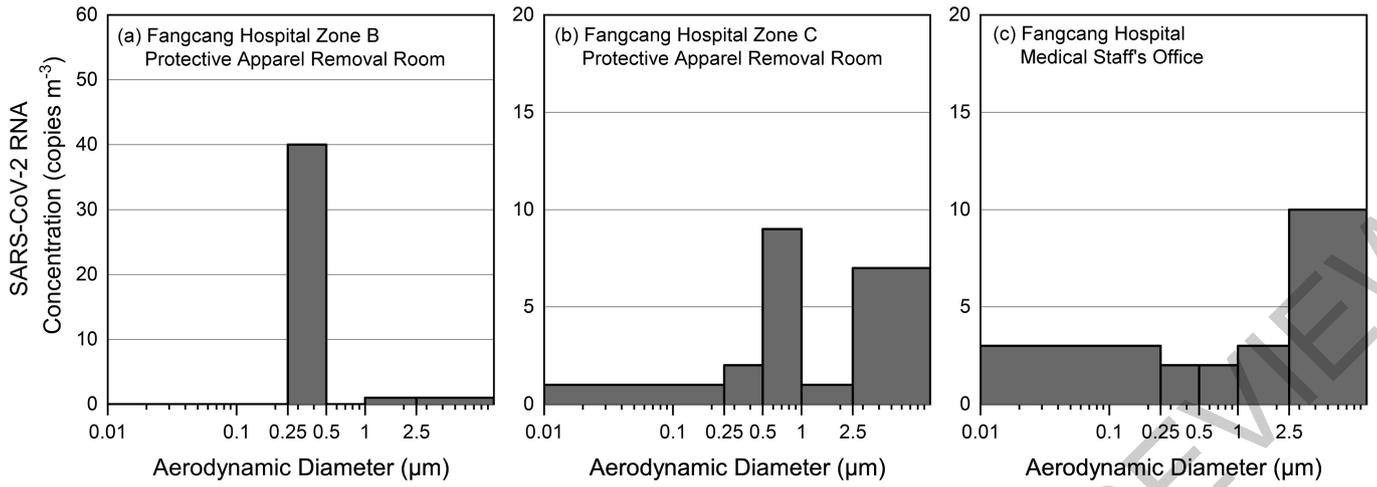


Fig. 1 | Concentration of airborne SARS-CoV-2 RNA in different aerosol size bins. The x axis represents aerodynamic diameter in logarithmic scale to cover the multiple magnitude of measured aerosol diameter..

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

Category	Sites	Sample Type	Concentration (copies m ⁻³)
Patient Areas (PAA)			
Fangcang Hospital	1. Zone A Workstation [#]	TSP ^a	1
		TSP ^b	9
	2. Zone B Workstation	TSP	1
	3. Zone C Workstation [#]	TSP ^a	5
TSP ^b		0	
	4. Patient Mobile Toilet Room	TSP	19
Renmin Hospital	5. Intensive Care Unit (ICU)	TSP	0
	6. Intensive Care Unit (ICU)	Deposition	31*
	7. Intensive Care Unit (ICU)	Deposition	113*
	8. Coronary Care Unit (CCU)	TSP	0
	9. Ward Zone 16	TSP	0
Medical Staff Areas (MSA)			
Fangcang Hospital	10. Zone A Protective Apparel Removal Room (PARR) [#]	TSP ^a	16
		TSP ^b	0
	11. Zone B Protective Apparel Removal Room (PARR)	Size Segregated	42
	12. Zone C Protective Apparel Removal Room (PARR) [#]	Size Segregated ^a	20
		TSP ^b	0
	13. Male Staff Change Room	TSP	20
	14. Female Staff Change Room	TSP	11
	15. Medical Staff's Office	Size Segregated	20
	16. Meeting Room	TSP	18
	17. Warehouse [#]	TSP ^a	21
TSP ^b		0	
Renmin Hospital	18. Passageway for Medical Staff	TSP	6
	19. Dining Room for Medical Staff	TSP	6
Public Areas (PUA)			
	20. Fangcang Hospital Pharmacy	TSP	3
	21. Renmin Hospital Doctor Office	TSP	0
	22. Renmin Hospital Outpatient Hall	TSP	0
	23. Renmin Hospital Outdoor	TSP	7
	24. University Office Doorside	TSP	0
	25. University Hospital Outpatient Hall	TSP	0
	26. Community Check Point	TSP	0
	27. Residential Building	TSP	0
	28. Supermarket	TSP	0
	29. Department Store 1	TSP	11
	30. Department Store 2	TSP	3
	31. Blank Control [#]	Field Blank ^a	0
		Field Blank ^b	0

* The reported values are virus aerosol deposition rate in copies m⁻² hour⁻¹.

Two batches of sampling were conducted for the sites. Detailed information is shown in Supplementary Table 1.

^a The samples taken during the first batch of sampling from Feb 17 to Feb 24, 2020.

^b The samples taken during the second batch of sampling on Mar 2, 2020.

Methods

1. Sample collection

The sampling was conducted between February 17 and March 2, 2020 in the locations by two batches as shown in Table 1. All aerosol samples were collected on presterilized gelatin filters (Sartorius, Germany). Total of 30 TSP aerosol samples were collected on 25 mm diameter filters loaded into styrene filter cassettes (SKC Inc., US) and sampled air at a fixed flow rate of 5.0 l per minute (LPM) using a portable pump (APEX2, Casella, US). Total of 3 size segregated aerosol samples were collected using a miniature cascade impactor (Sioutas impactor, SKC Inc., US) that separate aerosol into five ranges ($>2.5 \mu\text{m}$, 1.0 to 2.5 μm , 0.50 to 1.0 μm and 0.25 to 0.50 μm on 25 mm filter substrates, and 0 to 0.25 μm on 37 mm filters) at a flow rate of 9.0 LPM. Total of 2 aerosol deposition samples were 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 on the floor in two corners of Renmin Hospital ICU room intact for 7 days. Sampling durations and operation periods are detailed 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 floor. Considering the limited experimental condition with small sample size, the integrity and robustness of experiment protocol was examined extensively in the laboratory before the field sampling and described in Supplementary Table 2.

2. Analytical method and data analysis

After aerosol sample collection, all samples were processed immediately in the BSL-2 laboratory of Wuhan University. The 25, 37mm and 80 mm filter samples were dissolved in deionized water, then TRIzol LS Reagent (Invitrogen) was added to inactivate SARS-CoV-2 viruses and extract RNA according to the manufacturer's instruction. First strand cDNA was synthesized using PrimeScript RT kit (TakaRa). Optimized ddPCR was used to detect the presence of SARS-CoV-2 viruses following our previous study¹⁰. Analysis of the ddPCR data was performed with QuantaSoft software (Bio-Rad). The concentration reported by

the procedure equals copies of template per microlitre of the final 1x ddPCR reaction, which was normalized to copies m^{-3} in all the results, and hence the virus or viral RNA concentration in aerosol is expressed in copies m^{-3} hereafter. A detailed protocol is provided in 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 files, which include an additional Excel file containing Source Data for Fig. 1 and all raw data.

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Author contributions K.L., Y.C., Z.N., Q.Y.F., H.D.K. and K.F.H. conceptualized the study design; Y.L., Y.C., M.G., Y.L.L., and K.L. collected samples; Y.L., M.G., and X.J.L. did the laboratory tests; Y.L., Z.N., Y.C., N.K.G., M.G., X.J.L. and K.L. analysed the data; Y.C., Z.N., Y.L., Q.Y.F., H.D.K., J.C., K.F.H. and K.L. interpreted the results; Y.L. and Z.N. wrote the initial drafts of the manuscript; Y.L., Z.N., Y.C., and K.L. revised the manuscript; M.G., Y.L.L., N.K.G., L.S., Y.D., J.C., D.W., K.X., H.D.K., Q.Y.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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Give P values as exact values whenever suitable.
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Software and code

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Data collection

NONE

Data analysis

QuantaSoft analysis software v.1.7.4.0917 (Bio-Rad) was used for analysis of the ddPCR data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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Study description	This 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	Total of 35 distinct samples of three types, total suspended particle, size segregated and aerosol deposition were collected.
Sampling strategy	We sampled three types of virus aerosol samples at 30 sites covering patient and medical staff areas inside hospitals and in public areas in Wuhan. The sampling was designed to identify the hotspots of airborne SARS-CoV-2 and investigate their sources, and seek for evidences of their aerosol transmission across different isolation zones and air-surface transfer mechanisms.
Data collection	We collected two batches of samples from Feb 17 to Feb 24, 2020 and on Mar 2, 2020 respectively.
Timing and spatial scale	Sampling durations range from 5 to 20 hours for total suspended particle and size segregated samples and 7 days for aerosol deposition samples. Sampling air volumes range from 1.5 m ³ to 8.9 m ³ .
Data exclusions	No data were excluded in this work.
Reproducibility	The 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?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> 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/export	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.

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