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Temporal and Spatial Distribution of SARS-CoV-2 Aerosols in a Large-Scale Fangcang Shelter Hospital in Shanghai, China

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic caused by frequently mutating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had a worldwide impact. However, detailed data on the potential aerosol transmission of SARS-CoV-2 in real-world and controlled laboratory settings remain sparse. During the COVID-19 pandemic in Shanghai, China in 2022, samples were collected in a Fangcang shelter hospital, a large-scale temporary hospital rapidly built by converting the existing National Exhibition and Convention Center (Shanghai) into a health care facility. Aerosol samples at different sites and intervals around patients and in public areas, surface samples, and pharyngeal swab samples from corresponding patients were included. Samples were tested for SARS-CoV-2 using real-time quantitative polymerase chain reaction (RT-qPCR) assays, followed by sequencing if the cycle threshold (Ct) value was <30. The positivity rate for SARS-CoV-2 in aerosol samples was high in contaminated zones (37.5%, 104/277), especially around the bed (41.2%, 68/165) and near ventilation inlets (45.2%, 14/31). The prevalence of SARS-CoV-2 around the bed, public areas, and air inlets of exhaust vents fluctuated and was closely related to the positivity rate among patients at corresponding sampling sites. Some surface samples of different personal protective equipment from medical staff had high positivity rates. Sixty sequences of joined *ORF1ab* and spike genes obtained from sixty samples represented two main clusters of Omicron SARS-CoV-2. There was consistency in virus sequences from the same patient and their environment, and the detected virus sequences matched those of virus strains in circulation during the collection periods, which indicated a high likelihood of cross-contamination in the Fangcang shelter hospital. In summary, the results provide a quantitative and real landscape of the aerosol transmission of SARS-CoV-2 and a patient-centered view of contamination in large and enclosed spaces and offer a useful guide for taking targeted measures to avoid nosocomial infections during the management of SARS-CoV-2 or other respiratory virus diseases in a Fangcang shelter hospital.

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has imposed considerable public health and economic burdens worldwide. Emerging SARS-CoV-2 variants of concern, such as the Delta and Omicron variants, have been reported to have higher infectivity and transmissibility, possibly increasing the risk of transmission by aerosols (a colloid consisting of particles in a carrier gas with a diameter < 100 μm) [1–4], and have different epidemic characteristics [4]. Thus, aerosol transmission of the virus is a key and controversial issue [5–8]. At the beginning of the outbreak in 2020, neither the World Health Organization (WHO) nor the National Health Commission of the People's Republic of China recognized aerosol transmission of SARS-CoV-2 due to a lack of solid evidence. As early as 5 July 2020, 239 scientists from 32 countries, led by Morawska and Milton, wrote an open letter to the WHO, which was also published in an academic journal, emphasizing the importance of preventing the airborne transmission of COVID-19 [9].

The aerosol transmission of SARS-CoV-2 has been increasingly suggested by accumulating studies. The work by Dinoi et al. [10] clearly showed that airborne transmission could occur in poorly ventilated indoor areas or when infected individuals were present but was unlikely outdoors. In particular, the potential risk of indoor aerosol transmission was higher in places with a high density of people, such as hospitals [11]. Although mounting evidence suggests the airborne transmission of SARS-CoV-2, health advice has not caught up [12], and it was not until April 2022 that the WHO publicly reported the occurrence of aerosol transmission of SARS-CoV-2 [13]. This reflects both the complexity of SARS-CoV-2 and the scientific knowledge and understanding of a new virus is a process.

The presence of SARS-CoV-2 RNA detected by real-time quantitative polymerase chain reaction (RT-qPCR) in indoor aerosol samples has been widely reported in hospital wards [14–19], occasionally in nurse's stations [20], and even in a high-rise building via fecal aerosols [21], as well as in some other indoor public places and transportation facilities, including an airport, subways, and buses [22]. The presence of SARS-CoV-2 was also confirmed in outdoor aerosols before and during the first wave of the COVID-19 pandemic in three Swiss cities [23]. Additionally, a positive correlation was observed between SARS-CoV-2 RNA extracted and purified from fine particulate matter with aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) and the prevalence of COVID-19 in the local area [23]. Moreover, experimental results in animals have indicated a difference in aerosol transmission efficiency for different SARS-CoV-2 lineages or variants *in vivo* [24]. Higher amounts of SARS-CoV-2 were observed in the aerosols exhaled by some patients infected with the Omicron variant [25]. Simulation studies have provided probable routes of transmission of SARS-CoV-2 in a multistory building [26], a special building layout [27], a grocery store [28], a poorly ventilated courtroom [29], a classroom [30,31], and a concert hall [32]. Hospitalized patients with COVID-19 can shed SARS-CoV-2 into the environment [33]. All the abovementioned studies raise the important issue of defining the transmission mechanisms of SARS-CoV-2 and especially why some variants, such as Omicron, are so transmissible, something scientists are still struggling to understand [34].

These previous studies have mainly focused on local hospital environments or confined nonmedical places where patients and health personnel are co-located [14–20,26–32]. There are few studies on the effects of aerosol-generating procedures or the temporal and spatial distribution of aerosols in certain facilities, including large Fangcang shelter hospitals, which represent an important alternative strategy for community isolation of COVID-19 patients that has been adopted by many East Asian countries [35]. The viability of SARS-CoV-2 aerosols and their infectivity in the real world remain unclear. Only a few studies have reported on the recovery of infectious viruses from aerosol samples collected outside of a laboratory setting [36–39]. The infectivity of SARS-CoV-2 aerosols is expected to be mainly affected by multiple factors, such as the virus load of individual patients [40] and different SARS-CoV-2 variants, such as Omicron. Detection and collection of aerosol samples are affected by air sampling methods [41,42], sampling locations [18], and ventilation conditions [43]. Much more work is needed on the establishment of standard air sampling methods and their performance requirements [44].

Beginning in March 2022, the Omicron variant caused a large outbreak in Shanghai, China. Based on the successful experience with the Fangcang shelter hospital developed and used for the first time in China to tackle a COVID-19 outbreak in 2020 [45], the Shanghai Municipal People's Government converted the National Exhibition and Convention Center (NECC) into a Fangcang shelter hospital as one important measure to control the epidemic by isolating confirmed patients who were either asymptomatic or had mild to moderate symptoms. The term Fangcang, which sounds similar to Noah's Ark in Chinese, was borrowed from military field hospitals, but it refers to a novel concept: large, temporary hospitals built by converting public venues into health care facilities [46]. This large-scale hospital in Shanghai provided more than 46 000 beds and admitted 174 308 patients from 9 April to 31 May 2022. As a large temporary hospital, many of its medical staff and nonmedical workers (cleaning staff, security staff, deliverymen, administrative staff, police, volunteers, and venue maintenance staff) were on temporary assignment from different affiliated units. Thus, nosocomial infections and their control became a large and challenging issue. The risks of viral aerosol transmission in contaminated, semi-clean zones or even clean zones of the Fangcang shelter hospital and its neighboring environment became an important issue regarding the effectiveness of preventive measures and facilities.

Despite the significance of aerosols in the transmission of respiratory viruses, few studies have been conducted on the aerosol transmission risk of SARS-CoV-2; studies have focused mainly on aerosol virus quantification in a large-scale shelter or hospital for COVID-19 patients. The present study aimed to uncover how SARS-CoV-2 is dispersed with aerosols and to assess the possible aerosol transmission of SARS-CoV-2 and its influencing factors within a Fangcang shelter hospital. We focused on quantifying and monitoring the spatial and temporal aerosol SARS-CoV-2 load and analyzed the relationship between aerosolized SARS-CoV-2 and human infection as well as ventilation conditions in the Fangcang shelter hospital in Shanghai. The results herein provide a quantitative and real landscape of the aerosol transmission of SARS-CoV-2 in large and enclosed spaces, implying possible aerosol transmission risks and control measures for SARS-CoV-2 or other respiratory viruses within a large shelter.

2. Methods

2.1. Study site and patients

The study site was located at No. 333 Songze Avenue, Qingpu District, Shanghai, also called the "Four-Leaf Clover" Fangcang shelter hospital, which provided medical care, disease monitoring, food, shelter, and social activities for isolated patients with mild to moderate symptoms or no symptoms [46]. The Fangcang shelter hospital in this study was converted from the NECC and provided more than 47 000 beds distributed across 14 medical halls (eight on the first floor and six on the second floor; Fig. 1(a)). Each

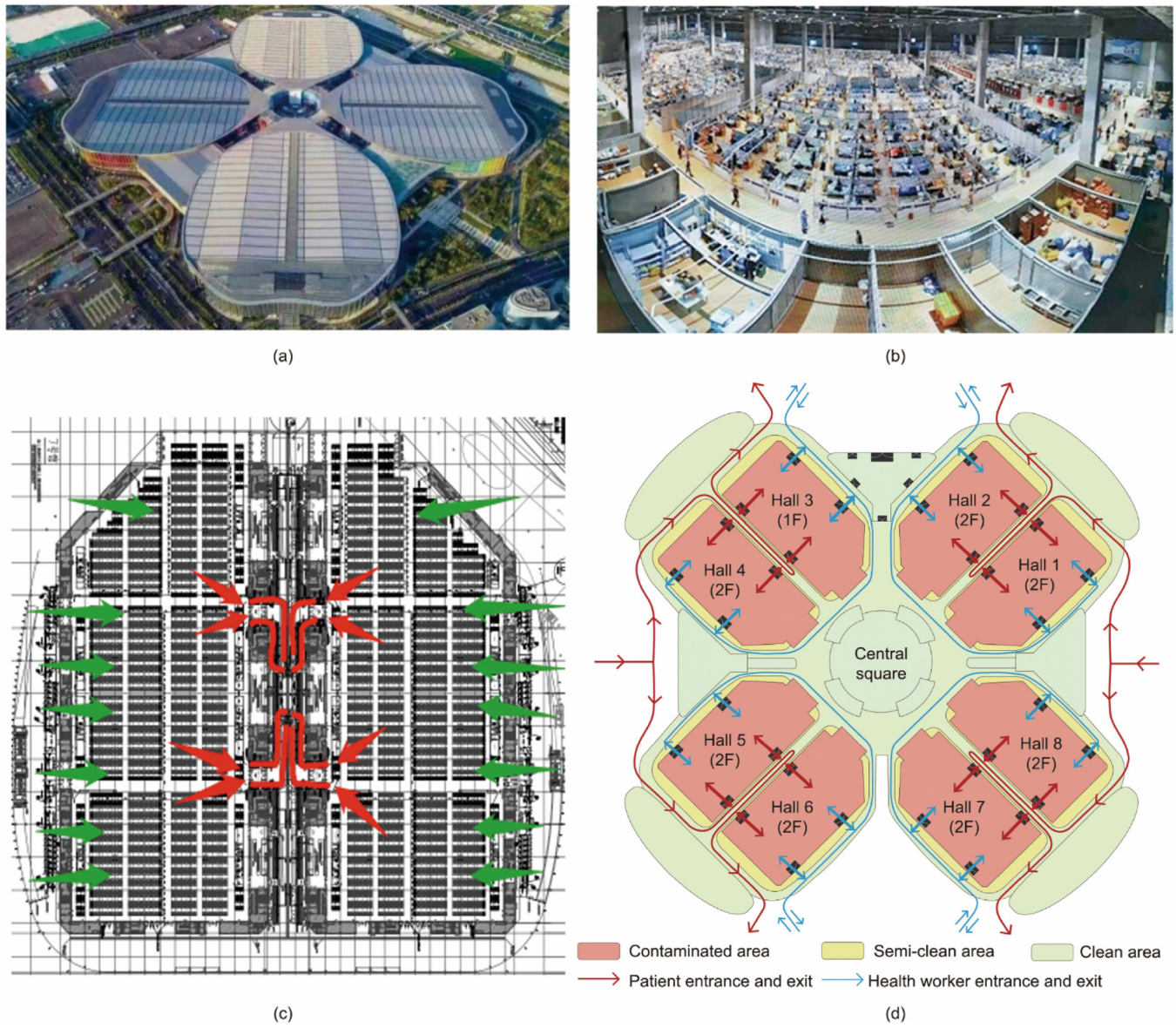

Fig. 1. Pictures of the "Four-Leaf Clover" Fangcang shelter hospital converted from the NECC. (a) Its shape resembles a four-leaf clover, giving it its name. (b) The structure of the medical hall, which covered an area of 26 520 m^2 and included 3000–3500 beds. (c) The designed ventilation mode in each hall with eight HEPA-filtered air exhausts placed close to the gate where the patient accessed the bathroom and the toilet. The opposite side received air by natural means. (d) Designed zones and passages in a Fangcang shelter hospital.

medical hall had a similar layout and structure, covering an area of 26 520 m^2 (270 m × 106 m) and including 3000–3500 beds (Fig. 1(b)), and had an estimated 424 320 m^3 of space based on dimensional measurement data. The ventilation system exhausted 496 000 $m^3 \cdot h^{-1}$ of air according to performance indicators from eight high-efficiency particulate air (HEPA)-filtered air exhausts (Fig. 1(c)), or a rate of 1.17 air changes per hour (ACH) per medical hall. All the air exhausts were stacked vertically at 2.5 m high and placed close to one side of the two patient entrances/exits to the toilet in the corridor between the two medical halls. Outdoor air was supplied from these gates and two openable widows in the opposite sidewall at a height of 8 m from the floor. The three-zone and two-channel temporary infectious disease hospital that met the criteria for the treatment of infectious diseases was constructed within 86 h (Fig. 1(d)). The hospital admitted 174 308 patients from the first round of patients admitted on April 9 to its closure on May 31 [47], accounting for approximately 25% of all reported cases during this outbreak in Shanghai. The age of the patients ranged from 1 to 92 years, with an average age of (41.50 ± 15.30) years. Among patients, 18.39% had other underlying diseases, and 22.14% were not vaccinated. The average length of stay was 7.3 d [47].

2.2. Sampling of aerosols and other environmental samples

Sampling sites covered 14 medical halls in the Fangcang shelter hospital from 23 April to 21 May 2022. Sites were distributed in three zones according to the degree of contamination: a clean zone (doctors' offices, material warehouse, ventilation system outlets outside all areas, air conditioner outlets, dressing rooms, and outdoor passageways), semi-clean zone (protective apparel removal rooms), and contaminated zone (doctor and nurse stations, areas near patients' beds, areas near the ventilation inlet (the intake through which the exhaust ventilation fan in the hall drew

polluted air), washing and lavatory areas for patients, and other public areas as well as trash transportation passages and areas in front of notice boards). A wetted wall cyclone sampler HRH-BSA350 bioaerosol sampler (Beijing Huironghe Technology Co., Ltd., China), was used to collect aerosol samples (Section S1 and Fig. S1 in Appendix A). This is a portable device with the ability to sample for long durations at a high flow rate (350 $L{\cdot}min^{-1}$). Pretests conducted by our research group estimated the device's cutoff size (D_{50}) at approximately 2.0 μm, and the enrichment ratio for collecting aerosolized coronavirus GX-P2V was $(3.9 \pm 0.5) \times 10^4$ min^{-1}, approximately (62.0 ± 7.9) times higher than that of the AGI-30 impinger, a widely used reference standard sampler [44,48].

We examined both spatial and temporal changes in the distribution of aerosol samples. For spatial analysis of aerosol samplers, two HRH-BSA350 devices were placed vertically at two different heights: 0.2 m, which represented the height at which active air and dust were potentially resuspended due to the active airflow induced by walking patients and staff; and 1.2 m, the average height of the head for a patient sitting on the bed. Aerosol sampling devices were placed 1.5 m away from a focal patient and facing toward their head or in the middle of groups of patients, where virus load (cycle threshold (Ct) value) information of the 10–12 patients 5–6 m away from the sampler was collected from medical records or tested by our research group. Aerosol samples at each site were collected every 10 min for 3500 L of air, which was estimated to be adequate for subsequent laboratory testing. For temporal analysis of aerosol samples, 13 sites in medical hall 1.2 were selected for longitudinal surveillance of dynamic changes in SARS-CoV-2 in aerosol samples. The sites chosen included the area near the inlet of air filters and two hospital bed areas that differed in overall viral loads of patients on May 14; three hospital bed areas on May 17; two testing sites and two doctor and nurse stations on May 19; and three other public areas and passages on May 21. The duration of sampling varied from 1.0 to 1.5 h to cover one complete exchange of air in a medical hall, based on the air change per hour (ACH) rate of 1.17. The PH-1 portable wind anemometer (Shanghai Longtuo Instrument Co., Ltd., China) was used to test the wind direction and speed in a passage in hall 1.2 for the duration of longitudinal sampling. Detailed sampling methods can be found in the equipment manufacturer's manual (Beijing Huironghe Technology Co., Ltd.). Briefly, 500 mL of phosphate-buffered saline (PBS) (Servicebio, China) was added to the fluid reservoir of the HRH-BSA350 device, and then the device was placed and turned on at the target location. When aerosol sampling was completed, aerosol particle samples were automatically collected in approximately 4 mL of PBS for subsequent analysis.

2.3. Other sample collection

When collecting aerosol samples of specific patients, corresponding pharyngeal swab samples of patients ("swab" samples) and surface swab samples ("surface" samples) for particulate pollution, organic pollutants, molecular pollution, ionic pollutants, etc., on the surface area of the mobile phones used by patients, the personal protective equipment (PPE) of health care workers, and the fan inlet grille for the ventilation equipment in the Fangcang hall were collected. Samples from masks used by patients ("mask" samples) were also collected. Virus sampling kits (Yocon Biotechnology Co., Ltd., China) that conserved the viability of viruses for later tests were used to collect surface samples and pharyngeal swabs. For masks, a 5 cm × 5 cm in the middle of each mask was cut out, cut into smaller pieces in the lab, placed into 15 mL tubes containing 5 mL of PBS and stored in a −80 °C freezer for subsequent testing.

2.4. Laboratory testing

All samples collected from the Fangcang shelter hospital were sent to a mobile BSL-3 laboratory to maintain biosafety. Viral RNA was extracted from each sample using an automatic magnetic ball nucleic acid extractor (KingFisher Flex; Thermo Fisher Scientific, USA) and a viral RNA extraction kit (DP438-T2F; TIANGEN, China) according to the manufacturer's instructions. Finally, RNA extracted from each sample was resuspended in 65 μL of elution buffer. RT-qPCR assays targeting both the structural nucleocapsid (N) protein and nonstructural (*ORF1ab*) genes of SARS-CoV-2 were performed using a novel coronavirus (2019-nCoV) nucleic acid detection kit (Beijing Kinghawk Pharmaceutical Co., Ltd., China) and the ABI7500 Fast Real-Time PCR System (Thermo Fisher Scientific).

2.5. Isolation of SARS-CoV-2 from samples

In vitro virus isolation was performed for swab samples with a Ct value < 25 and for aerosol samples with a Ct value < 30. To mix high-concentration antibiotics (final 10×) with the mucus-rich swab samples and naturally separate the virion coverings from virions, samples were incubated with a penicillin–streptomycin antibiotic mix (Thermo Fisher Scientific), keeping a final concentration of 1000 $U{\cdot}mL^{-1}$ (10× solution) at 4 °C for 2 h. These samples were then diluted (1:2, 1:5, or 1:10) with Dulbecco's modified essential medium (DMEM; Gibco, USA) and 0.2% bovine serum albumin (BSA; Sigma-Aldrich, USA), keeping a final concentration of 500, 200, or 100 $U{\cdot}mL^{-1}$. The samples in DMEM were inoculated onto 85% confluent Vero-E6 cells (American Type Culture Collection, USA), and the cells were subsequently incubated for 2 h in 5% CO_2 at 37 °C, followed by three rounds of washing with warm PBS, after the removal of the incubated samples, and then updated with DMEM supplemented with 2% FBS or 0.2% BSA for further incubation in 5% CO_2 at 37 °C. Detached cells were also resuspended in the medium in each cell well. To evaluate possible virus growth, the supernatant of incubated cells was collected at 0, 48, or 72 h post-incubation for quantification of SARS-CoV-2 RNA. A Ct value reduction threshold of 2 was used as the criterion for virus growth. At 96 h post-incubation, all incubated samples were subjected to another round of virus isolation regardless of the virus growth result in the first test. Virus isolation was performed for the samples with a titer multiplication of more than 10^4 in viral RNA via RT-qPCR, and a positive test for the virus spike antigen was performed using the colloidal gold method (Amazing Biotech (Shanghai) Co., Ltd., China).

2.6. Virus sequencing of SARS-CoV-2 in the environment

Next-generation sequencing was used for positive samples with low Ct values (< 30). The library was constructed using the QIA seq system and then sequenced using an Illumina MiSeq. The sequencing data were filtered using Fastp and CLC Genomic Workbench V21. Filtered reads were mapped to the SARS-CoV-2 reference genome (GenBank accession number NC_04512.2) with CLC Genomic Workbench V21. The phylogenetic tree was constructed for the *ORF1ab* and spike genes for 60 sequence samples, together with 12 reference sequences (Table S1 in Appendix A) from patients in Shanghai at the same time, using IQ-TREE with 1000 bootstrap iterations as previously reported [49].

2.7. Ethical considerations

This work was conducted as part of the surveillance and public health response to contain the COVID-19 outbreak in Shanghai. The activities were coordinated by the NECC Fangcang shelter hospital.

The requirement for informed consent from patients was waived. All information regarding individuals was anonymized in the report.

2.8. Data analyses

Sample sites were referenced and linked to a map of the medical halls according to patient bed codes. Test data processing was carried out following the instructions of the PCR kit, setting thresholds for each gene and evaluating the presence of suitable PCR curves. The results are expressed as Ct values for each detected target. The quantification curves were determined using the SARS-CoV-2 RNA standard (National Institute of Metrology China, GBW(E)091089; Fig. S2 in Appendix A). According to a previous report [50], the RNA concentration (virus load) of the aerosol samples (copies·m^{-3}) for the *ORF1ab* gene was calculated as:

$$C_1 = \frac{10^{(46.09-\mathrm{Ct})/3.321} \times 4\ \mathrm{mL}\ (\text{total volume of transport medium})}{3500\ \mathrm{L}\ (\text{air volume by sampler in 10 min})} \times 10^3\ \left(\mathrm{L \cdot m^{-3}}\right)$$

The RNA concentration (virus load) of the aerosol samples (copies·m^{-3}) for the *N* gene was calculated as follows:

$$C_2 = \frac{10^{(46.27-\mathrm{Ct})/3.383} \times 4\ \mathrm{mL}\ (\text{total volume of transport medium})}{3500\ \mathrm{L}\ (\text{air volume by sampler in 10 min})} \times 10^3\ \left(\mathrm{L \cdot m^{-3}}\right)$$

Descriptive statistics were calculated for all variables. Continuous variables were summarized as means ± standard deviations (SDs). Categorical variables were summarized as frequencies and proportions. To estimate the statistical significance of the differences between groups, a χ^2 test or Fisher's exact test was used, as appropriate. A two-sided *P* value of less than 0.05 was regarded as statistically significant. All statistical analyses were conducted with SPSS software (version 18.0). When analyzing the correlations among variables, Spearman's correlation coefficients were used to measure the correlations, with $P < 0.05$ indicating statistical significance.

3. Results

3.1. Overall prevalence of SARS-CoV-2 in the Fangcang shelter hospital

From 23 April to 21 May 2022, 710 aerosol samples, surface samples, pharyngeal swabs, and mask samples were collected, and 287 (40.4%) of these returned positive RT-qPCR results. The range of positivity rates for all aerosol samples and surface samples each day varied from 0 to 100.0% (Fig. S3 in Appendix A). The positivity rates of samples among medical halls differed by location ($P < 0.001$; Table 1). The positivity rates of aerosol samples (105/347, 30.3%), surface samples (93/208, 44.7%), pharyngeal swabs (77/127, 60.6%), and mask samples (12/28, 42.9%) also differed significantly ($\chi^2 = 38.074$, $P < 0.001$; Table 2). All 29 aerosol samples collected from the clean zone (outside of the medical hall) were negative for SARS-CoV-2 RNA. In the semi-clean zone, only one (2.4%) out of 42 aerosol samples tested positive, which was collected from a corridor modification room used for removing outer protective clothing, located outside the contaminated zone in hall 1.2. Samples taken from contaminated zones had a relatively high prevalence of SARS-CoV-2 RNA (37.5%) detected in aerosols from contamination zone (Table 2). The isolation of viable SARS-CoV-2 virus from one patient swab sample was successful but not from any aerosol samples (Fig. S4 in Appendix A).

Table 1
Number and positivity rate of SARS-CoV-2 from samples collected in the Fangcang shelter hospital by RT-qPCR assay.

Hall code	Test No.	Positive No.	Positivity rate (%)
1.1	27	10	37.0
1.2	232	81	34.9
2.1	5	1	20.0
2.2	260	140	53.8
3.1	13	3	23.1
4.1	11	5	45.5
5.1	42	22	52.4
5.2	7	0	0
6.1	11	2	18.2
6.2	20	8	40.0
7.1	14	0	0
7.2	42	14	33.3
8.1	9	0	0
8.2	17	1	5.9
Total	710	287	40.4

Fisher's exact method results of samples of different halls: $P < 0.001$.

Table 2
Test results of different sample types collected from the Fangcang shelter hospital in Shanghai.

Sample types	Tested No.	Positive No.	Positivity rate (%)
Aerosol samples			
Semi-clean zone	41	1	2.4
Clean zone	29	0	0
Contaminated zone	277	104	37.5
Surface samples	208	93	44.7
Pharyngeal swabs	127	77	60.6
Masks of patients	28	12	42.9
Total	710	287	40.4

χ^2 test result of different samples types: $\chi^2 = 38.074$, $P < 0.001$.

3.2. Spatial distribution of SARS-CoV-2 RNA detected in the contaminated zone

In the contaminated zone, aerosol samples, surface samples of mobile phones, mask samples, and pharyngeal swabs all had a high prevalence of SARS-CoV-2, although they differed significantly from each other ($\chi^2 = 18.812$, $P < 0.001$; Table 3). The overall positivity rate in aerosol samples was 37.5% (104/277), with a higher prevalence in bedside areas (41.2%) and ventilation inlet areas (45.2%), followed by public areas for patients (30.6%) and doctor and nurse stations (24.4%) (Table 3). Public areas for patients included passages between bed units, refuse transfer stations, boiler rooms, in front of notice boards and washrooms. The positivity rates of samples from ventilation inlets varied by hall; halls 1.1, 6.2, and 8.2 all had negative results (0/7), while halls 2.2 and 6.1 all had positive results (5/5). The longitudinal surveillance of hall 1.2 had a positivity rate of 54.5% (12/22), and the overall positivity rate of pharyngeal swabs in contaminated zone was 60.6%. Among the environmental samples, the highest positivity rate came from object surface samples (44.9%), followed by masks of patients (42.9%).

Aerosol samples from different heights did not differ significantly ($\chi^2 = 2.534$, $P = 0.111$); the positivity rates were 52.0% at 0.2 m and 37.9% at 1.2 m (Table 4). Aerosol samples collected near patients with viral load test Ct values < 35 had positivity rates that reached 60.9% (42/69) ($\chi^2 = 25.817$, $P < 0.001$; Table 5). Correlation analysis showed that virus loads of aerosol samples were negatively correlated with Ct values of swab samples from the corresponding patients (Spearman's correlation coefficient (r_s) = −0.379; $P = 0.035$; Fig. 2(a)). However, the Ct values of aerosol samples were not significantly correlated with the mean Ct values

Table 3
Test results of SARS-CoV-2 among different types of samples in contaminated zones by RT-qPCR.

Types	Tested No.	Positive No.	Positivity rate (%)	χ^2	*P*
Surface samples					
Doctor and nurse stations	2	1	50.0	—	< 0.001
Bedside areas	116	63	54.3	—	—
Public areas for patients	2	2	100.0	—	—
Ventilation inlets	3	3	100.0	—	—
Floating medical staff	84	24	28.6	—	—
Aerosol samples					
Doctor and nurse stations	45	11	24.4	5.757	0.126
Bedside areas	165	68	41.2	—	—
Public areas for patients	36	11	30.6	—	—
Ventilation inlets	31	14	45.2	—	—
Mask samples					
Bedside areas	28	12	42.9	—	—
Pharyngeal swabs					
Bedside areas	127	77	60.6	—	—
Total	639	286	44.8	—	—

χ^2 test result of different sample types in contaminated zone: $\chi^2 = 18.812$, $P < 0.001$; rows with only *P* value results were analyzed using Fisher's exact test.

Table 4
Test results for SARS-CoV-2 among aerosol samples collected at different heights in the contaminated zone by RT-qPCR.

Height (m)[a]	Tested No.	Positive No.	Positivity rate (%)
0.2	25	13	52.0
1.2	140	53	37.9
1.2[b]	112	38	33.9
Total	277	104	37.5

[a] Placing the instrument's air intakes at different heights.
[b] Longitudinal sampling sites and uncertain specified target sites.

of swab samples from the nearest 11–13 patients (including the corresponding patient) in a circular range around the aerosol sample collection site ($r_s = 0.260$, $P = 0.158$; Fig. 2(b)). Furthermore, we mapped all the negative and positive aerosol samples collected in halls 2.2 (Fig. S5(a) in Appendix A) and 1.2 (Fig. S5(b) in Appendix A). Additionally, a possible influence of the airflow in the Fangcang shelter hospital on the aerosol virus load was analyzed. Spearman's correlation between the aerosol virus load and the distance from either air ventilation inlet was not significant in hall 1.2 ($r_s = 0.150$, $P = 0.520$; $r_s = -0.140$, $P = 0.530$) (Fig. 2(c)). A nonsignificant Spearman's correlation was also observed in hall 2.2 ($r_s = -0.250$, $P = 0.090$; $r_s = 0.010$, $P = 0.960$) (Fig. 2(d)).

3.3. Dynamic distribution characteristics of SARS-CoV-2 aerosols in the contaminated zone

Among 13 sites in medical hall 1.2 (Fig. 3(a)), SARS-CoV-2 aerosol concentrations in air ventilation inlets varied with time (Fig. 3(b)). For the two patient units sampled on May 14 (Fig. 3(b)) and three patient units sampled on May 17 (Fig. 3(c)), ten patients around the air sampling sites also underwent pharyngeal swab sampling and testing. Of the ten patients tested in each area, three in unit 0517-D3 were positive, one in unit 0517-F1 was positive, and one in unit 0517-F4 was weakly positive (Ct > 35). Aerosol sample results were consistent with those of surrounding patients. The area with a greater number of positive patients and a lower average Ct value (0517-D3) had higher aerosol positivity rates, and the area with fewer positive patients and a higher average Ct value (0517-F1) had only negative results from SARS-CoV-2 aerosol sample tests. In addition, nurse station No. 6 and patients' routine pharyngeal swab centralized sampling site A, where air sterilization interventions (air purifier or electric fan) were in use, showed negative results (Fig. 3(d)). In a temporary poker-playing public area (0521-E4) for patients who had negative results and were waiting to be discharged and in walking passages, all samples collected at six longitudinal time points were negative (Fig. 3(e)). During sampling, wind speed varied from 0–3.0 $m \cdot s^{-1}$, with an unstable direction (Table S2 in Appendix A).

3.4. SARS-CoV-2 contamination of patients' masks and medical workers' PPE

A 64.3% (9/14) positivity rate was observed for masks worn by patients (Table 6). However, time spent wearing the mask was independent of positivity rate. Most patients wore masks for a relatively long time, with a mean of (6.84 ± 4.07) h. Regarding SARS-CoV-2 contamination on the PPE of medical workers, the highest positivity rate was for samples from shoe covers, followed by boot covers, the back of the suit, and the chest of the suit. These PPE location differences were statistically significant ($P < 0.0001$; Table 6).

Table 5
Test results of SARS-CoV-2 nucleic acid of aerosol samples around patients with different viral loads (Ct value).

Aerosol samples (*n* (%))	Ct value			Unclear[a]	Total (%)
	<35	35–40	> 40		
Negative	27 (16.4)	11 (6.7)	47 (28.5)	12 (7.3)	97 (58.8)
Positive	42 (25.5)	7 (4.2)	9 (5.5)	10 (6.1)	68 (41.2)
Total	69 (41.8)	18 (10.9)	56 (33.9)	22 (13.3)	165 (100.0)

χ^2 test results of aerosol samples of different Ct value: $\chi^2 = 25.817$, $P < 0.001$.
[a] Longitudinal collection of samples in fixed site, Ct value of corresponding patients were not recorded and tested.

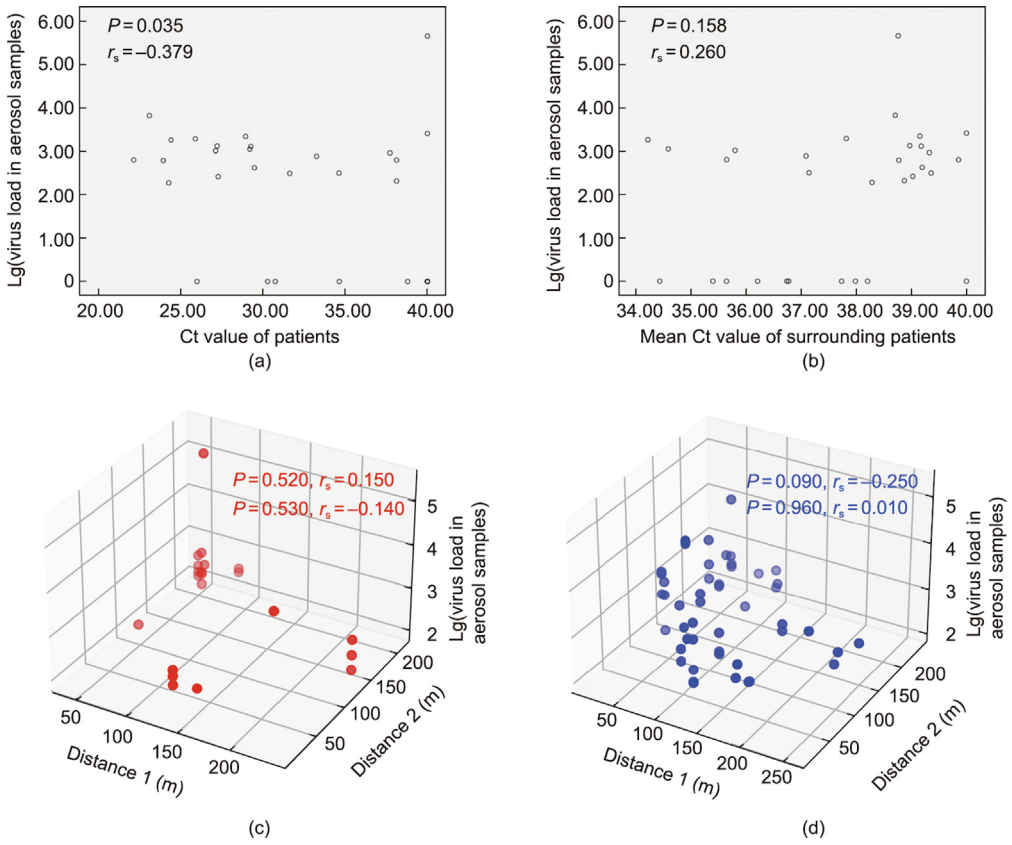

Fig. 2. Correlation analysis between positive aerosol samples and corresponding patients. (a) Scatter plot of virus loads in aerosol samples and corresponding patients, with correlation analysis. (b) Scatter plot of virus loads and mean Ct value of corresponding patients and the nearest 10–12 patients around the sample site center, with correlation analysis. (c) Scatter plot of virus loads in aerosol samples and the distance of sampling sites from either air ventilation inlet in hall 1.2. (d) Scatter plot of virus loads in aerosol samples and the distance of sampling sites from either air ventilation inlet in hall 2.2, with correlation analysis.

3.5. Molecular epidemiological analysis of SARS-CoV-2 in the environment

We acquired 60 SARS-CoV-2 sequences, among which 11 were from aerosol samples, 19 from surface samples, 28 from patient swabs, and 2 from a mask sample. All were SARS-CoV-2 Omicron variant sequences. A phylogenetic tree showed that samples formed two main clusters. Cluster one was dominated by samples collected before May 9 (lower half of branch in Fig. 4). After that day, a different strain appeared, and the two strains coexisted, with the second strain gradually becoming more dominant and eventually forming its own branch (upper half of branch in Fig. 4). Sequences obtained from aerosol samples in halls 1.2 and 2.2 were clustered with swab samples taken the same day in those halls. In addition, air, swab, and mask samples from case D3-149 were similar. In samples from case F1-021, sequences from swab and mask samples were closely related but differed from the surface sample of the patient's mobile phone. Most swab samples were randomly distributed among clades, and it was thus clear when swab samples from a father and son (Family 1, D1-139, and D1-125) and a couple (Family 2, E5-038, and E5-040) were closely related to each other (Fig. 4).

4. Discussion

This study revealed the temporal and spatial characteristics of SARS-CoV-2 aerosols in a large-scale Fangcang shelter hospital and provided a quantitative and real landscape of the aerosol transportation of respiratory tract-transmitted viruses in large and enclosed spaces. SARS-CoV-2 load quantification of the samples from the patient and his or her surroundings as well as from ventilation inlets indicated a patient-centered gradient of decreasing virus contamination under the condition of emergency modification and insufficient ventilation. The results are useful to guide the adoption of large shelters as a means of isolation and management of mild and asymptomatic patients with COVID-19 or other respiratory diseases by improving ventilation efficiency and decentralized management of patients.

No SARS-CoV-2 aerosol contamination was detected in any samples from the clean zone, aerosol contamination in the semi-clean zone was minimal, and there was a high prevalence of SARS-CoV-2 in aerosol samples from the contaminated zone. This confirms the relative effectiveness of the "three zones and two passages" layout (Fig. 1(d)), implementation of personal protective and preventive procedures, environmental disinfection procedures, and infection control measures. However, the comprehensive surveillance of the Fangcang shelter hospital, in particular the results of aerosol samples, suggests that the contaminated zone should be further divided into high-, medium- and low-risk areas. The high-risk areas included bedside areas (≤1.5 m from bed), mobile phone surfaces of patients with high viral loads, and ventilation inlets. Medium-risk areas included public areas where patients gathered. According to a study, air purifiers can be used to minimize the potential risks associated with aerosols in a high-risk environment [51]. Therefore, low-risk areas were doctor and nurse stations, which may be partially explained by the use of air purifiers (MKJ4000; Jiaxing Heyu Purification Technology Co., Ltd., China; Y-SB9101/CMCS-02B; China Electronics Technology Group Corporation, China), high-power fans, regular cleaning of

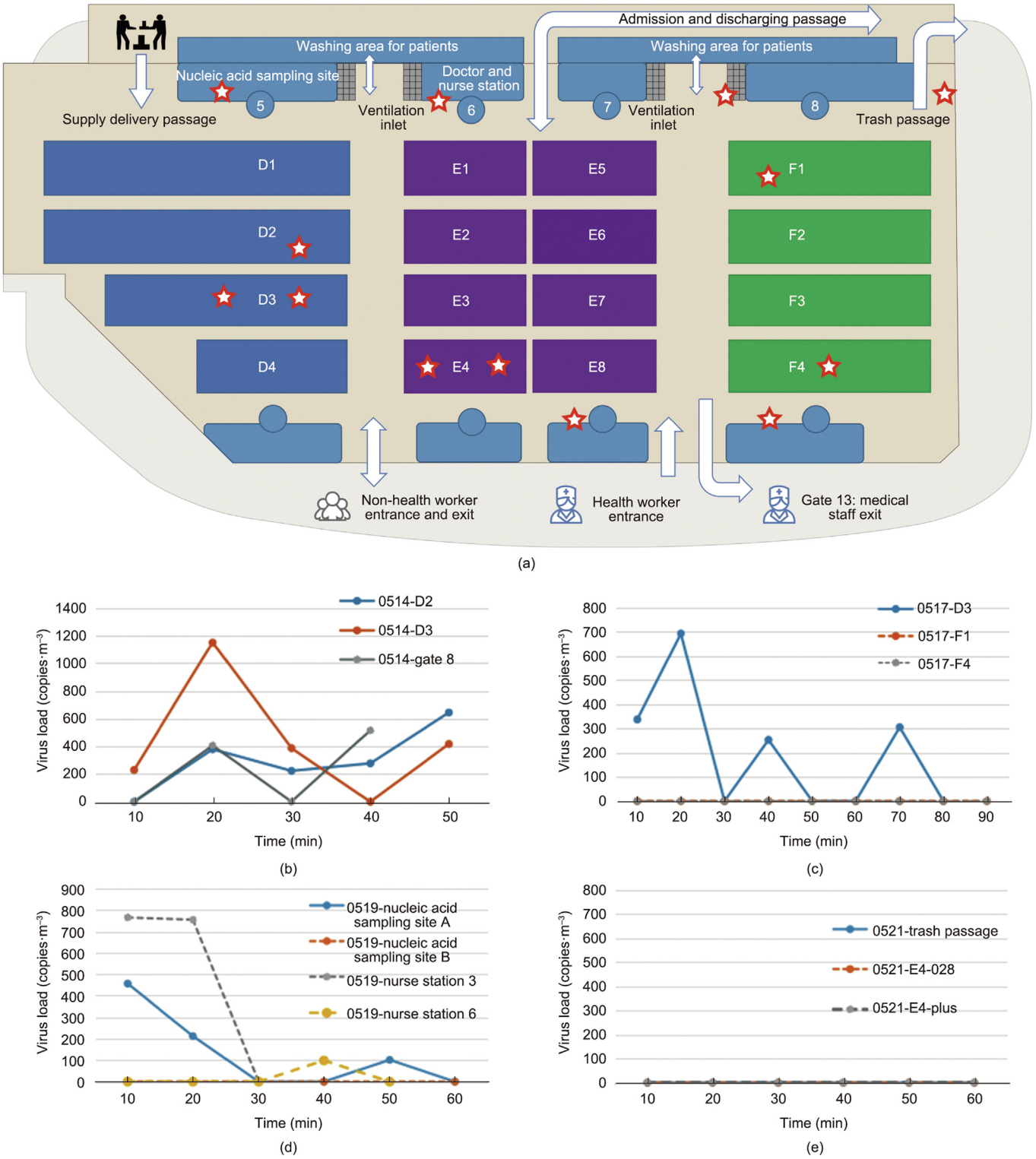

Fig. 3. Dynamic distribution of aerosols in the contaminated zone. (a) Sampling sites in medical hall 1.2 used for longitudinal surveillance to monitor the dynamic change in SARS-CoV-2 aerosols; the red stars show the sampling sites. (b) Dynamic distribution of positive samples collected at air filter inlets and two hospital bed areas on May 14. (c) Dynamic distribution of positive samples collected in three hospital bed areas on May 17. (d) Dynamic distribution of positive samples collected from the patient pharyngeal swab sampling site and doctor and nurse stations on May 19. (e) Dynamic distribution of positive samples collected in other public areas and passages on May 21.

surfaces, and good personal disinfection habits of health care workers. The positivity rate of pharyngeal swabs was 60.6%. Although sampling error cannot be ruled out, this result is expected because 60% of patients in the hospital were asymptomatic, and it was common for some patients who were negative that day to test positive again on the next or the third day. The production, release, and transport of respiratory aerosols are closely related to human respiration and coughing, airflow, air circulation, and the physical properties of the aerosols themselves. Therefore, newly admitted patients should be separated as much as possible

Table 6
Contamination results of masks worn by patients and the surface of PPE used by medical workers.

Test results	Negative (*n* (%))	Positive (*n* (%))	Total (*n* (%))	Positivity rate (%)	χ^2	*P*
Ct of patients						
< 35	5 (17.86)	9 (32.14)	14 (50.00)	64.3	5.250	0.022
> 35	11 (39.29)	3 (10.71)	14 (50.00)	21.4	—	—
Time of wearing masks (h)						
< 4	5 (17.86)	2 (7.14)	7 (25.00)	28.6	—	0.688
> 4	11 (39.29)	10 (35.71)	21 (75.00)	47.6	—	—
PPE of medical workers						
Goggles	12 (14.29)	0 (0)	12 (14.29)	0	—	< 0.0001
Chest of suit	9 (10.71)	3 (3.57)	12 (14.29)	25.0	—	—
Upper limbs of suit	11 (13.10)	1 (1.19)	12 (14.29)	8.3	—	—
Back of suit	8 (9.52)	4 (4.76)	12 (14.29)	33.3	—	—
Boot cover	7 (8.33)	5 (5.95)	12 (14.29)	41.7	—	—
Shoe cover	2 (2.38)	10 (11.90)	12 (14.29)	83.3	—	—
Gloves	11 (13.10)	1 (1.19)	12 (14.29)	8.3	—	—

Rows with only *P* value results were analyzed using Fisher's exact test.

among inpatient beds. Medical staff should try to keep a distance of at least 1.5 m when talking with patients face to face and minimize their time in ward areas or other crowded patient areas to ensure the filtration function of masks. It is advised that air purification devices with HEPA sterilizer filters be used in contaminated zones. Public health education, especially on the disinfection of mobile phones for patients, is also recommended.

Airborne transmission of SARS-CoV-2 and its variants has been confirmed in animal models such as ferrets and hamsters [52,53]. It has been validated that SARS-CoV-2 can be transmitted between hamsters as < 5 μm small particle aerosols at a distance of 2 m [24]. The occurrence of SARS-CoV-2 nosocomial infections in health care settings reveals the real possibility of aerosol transmission among humans [54]. An experimental study has shown that COVID-19 patients in earlier disease stages exhaled millions of SARS-CoV-2 particles per hour, and intact SARS-CoV-2 virus particles that are infectious and replicable can be found in aerosol samples filtered to < 1 μm collected near patients and that the coronavirus can survive in aerosols for up to 3 h [4]. The correlation analysis also indicates a higher risk of SARS-CoV-2 aerosol transmission around patients and suggests that positive aerosol samples were mainly caused by the target individual. Presumably, the air around a target patient is less likely to be affected by surrounding patients, and the compartment setting in the Fangcang shelter hospital was useful in isolating and quarantining patients.

We also found high positivity rates for surface swab samples from the anterior chest area of protective clothing and boot covers. In the Fangcang shelter hospital, all patients were required to wear uniformly issued surgical masks, which are effective in preventing droplet transmission, and were not allowed to remove their masks, especially when dealing with other people and health care workers, except when swab samples were collected in specific areas. It is not common for medical staff to touch their clothing, especially in front of chest and boot covers, which are not easily touched physically, after working with patients. These contaminated parts are thus likely to have come from aerosols.

The air ventilation mode has a great influence on the transport of aerosols, notably diffusion. Although stratum ventilation was a good design at first, the Fangcang shelter hospital in Shanghai finally used a hybrid mechanical–natural system for mixed ventilation, which was supposed to fully mix the pollutants and remove them through a diluting ventilation process [55]. The outdoor air was supplied from two patient entrances and two openable widows in the opposite sidewall at a height of 8 m from the floor. The rate of ACH was low at 1.17 in the halls of the Fangcang shelter hospital. The wind speed varied from 0 to 1.6 $m \cdot s^{-1}$ with no consistent direction. This low ACH rate and wind speed may have allowed a greater accumulation of virus-laden particles near patients, which can explain the high virus positivity rates found in aerosol samples from near patients. According to previous studies, in still air, it takes 12.2 h for an aerosol with a diameter of 1 μm to fall to the ground from a height of 1.5 m [37], and the time required for removing 90% and 99% airborne contaminants under ACH conditions is approximately 138 and 276 min, respectively [56], which indicates that small particles can persist suspended in indoor air for hours before being removed and distributed uniformly in the occupant zone according to the mixing ventilation mode. In this study, the outdoor air was supplied from two patient entrances and two openable widows in the opposite sidewall. The indoor air was discharged mechanically by eight air exhausts. Hall 2.2 showed a relatively homogeneous distribution tendency for aerosol samples, and the different patient areas differed significantly in average virus loads, while the positive aerosol samples from hall 1.2 for dynamic distribution analysis were not uniformly distributed. These results indicate that SARS-CoV-2-laden aerosols settled nearby when produced by patients. It may also be mainly caused by the extraordinary capacity of the halls with a height of 16 m, in which aerosols can be adequately diluted, and the possibility increased that the viral concentration in air is below the detection limit of the sampler. The different spatial and temporal viral aerosol distribution situations among the halls reveal the complexity of pathogen air transmission mechanisms.

The results demonstrate the potential for close-range aerosol transmission in the hospital. This phenomenon may be caused by two factors. First, the hall had a relatively low ventilation rate, and the rate of ACH was 1.17. A previous study proved that a low ACH ventilation condition increases the aerosol concentration in the source room for a short time in the beginning and may raise the near-field short exposure risk [57]. Second, the surgical masks worn by patients partially impair the horizontal velocity and quantity of exhaled aerosols [58], which may subsequently decrease the aerosol transmission distance and virus concentrations in air.

The results of phylogenetic analysis revealed the molecular connection between samples. Sequences of aerosol samples and swab samples taken the same day in those halls indicated that admitted patients were shedding virus into the air. In addition, the similarity of different samples from the same case proved that samples taken from the same patient were consistent with each other. In addition, the difference in surface samples of patients' mobile phones from their swab and mask samples suggested that the phone surface possessed viruses of different origins, elevating the risk of cross-contamination and infection. Interestingly, swab samples of cases of relatives (related or spouse) revealed the likelihood of being exposed and infected with the same strain of virus (Fig. 4). These results confirm the dissemination of two different strains of highly diverse Omicron SARS-CoV-2 in the Fangcang shelter hospital. It

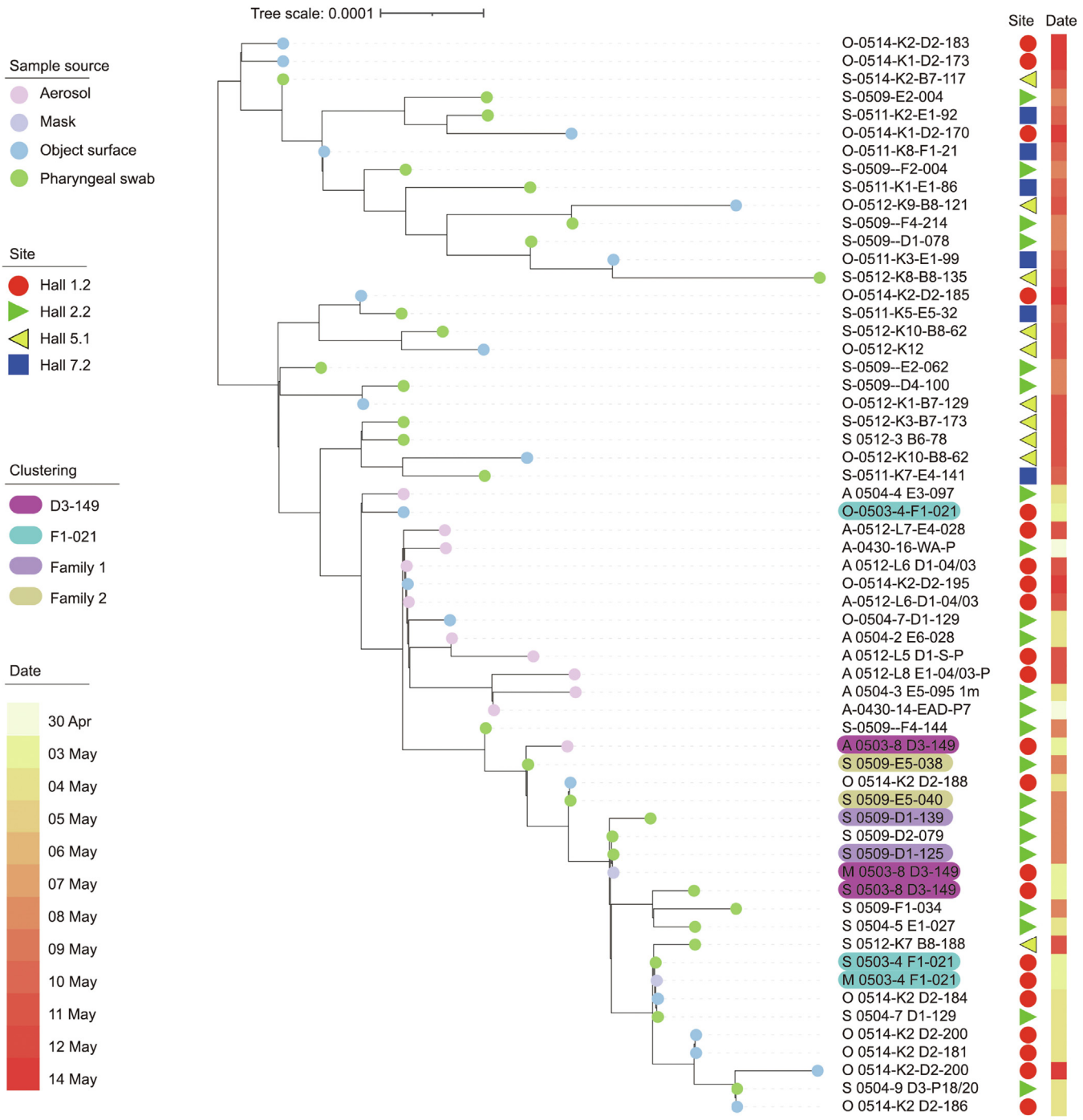

Fig. 4. Phylogenetic analysis of 60 virus sequences from the Fangcang shelter hospital. The phylogenetic tree was constructed using IQ-TREE with 1000 bootstrap replicates. Different colored circles in the tree represent different sample sources (aerosol (A), mask (M), object surface (O), or pharyngeal swab (S)). Highlight colors for sample identifier codes identify four groups of samples that are related to each other ("Clustering" in the legend). Different shapes on the right indicate the sampling site, and the sampling date is indicated by the progressive color bar at the far right.

also confirms the characteristics of gene mutations at specific sites and provides molecular evidence of the transportation route of SARS-CoV-2 among humans, masks, surfaces, and air. The in-depth analysis of mutations suggests that cross-transmission is likely.

This study has several important limitations. First, virus isolation for SARS-CoV-2 from aerosol samples was unsuccessful. The survival of the virus in aerosols reported by previous studies showed that humidity and temperature can affect the transmission efficiency of virus-laden aerosols by controlling virus infectivity and droplet suspension time in the air, which differs by the diameter of aerosols [59,60]. Unsuccessful isolation of the virus may have been due to the very low viral concentration in the aerosol and the viability of the virus caused by humidity and temperature or to virus damage caused by impact forces in the sampler. Second, the buoyant thermal plume of patients may have caused aerosol uplift, forming a high-concentration area at the top of the hall. Since there was extraordinary capacity in the hall with a height

of 16 m and the air sampling strategies did not include vertically high location sampling, whether there was a higher concentration area near the ceiling is in need of better verification, which could be evidence of the airflow pattern assumption. In addition, the sampling duration should be prolonged for low concentrations. Third, we did not consider other risk factors that could influence aerosol dispersion, such as temperature, light, humidity, and air circulation. Therefore, we cannot rule out the possibility that other factors could be important for aerosol distribution or viral activity. Fourth, as the length of stay in the hospital for each patient was not recorded, the phase of each infected patient and the average duration of virus shedding of each patient were not discussed in this study. Last, the low efficiency of the sampler and cutoff value of 2 μm may have resulted in the underestimation of the levels of airborne virus. These limitations, however, do not reduce the usefulness of the findings that the temporal and spatial distribution characteristics of SARS-CoV-2 aerosols depend on the relevant patient factors and ventilation facilities that can be improved in a large-scale Fangcang shelter hospital.

In sum, Omicron SARS-CoV-2 has the clear potential to be transmitted through aerosols, although the infectivity of the aerosolized virus detected in the Fangcang shelter hospital was not established. This study first provided a quantitative and real landscape of the aerosol transport of SARS-CoV-2 and a patient-centered gradient of decreasing virus contamination in large and enclosed places, and offered a useful guide for taking targeted measures to avoid nosocomial infection during the management of SARS-CoV-2 or other respiratory virus diseases in a Fangcang shelter hospital.

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Compliance with ethics guidelines

Jiafu Jiang, Zhe Yin, Jing Li, Leili Jia, Rulin He, Wenhui Yang, Jihu Yang, Hang Fan, Sen Zhang,Yunfei Wang, Zengming Zhao, Haoran Peng, Lizhong Li, Yi Yang, Shiyong Fan, Rong Xiang, Jianshu Guo, Jin-Jin Wang, Juanning Wei, Fengling Zhou, Ding Liu, Ping Zhao, Yujun Cui, Yunxi Liu, Dongsheng Zhou, and Gang Dong declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2023.06.006.

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