Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Original Article

Outbreak investigation in a COVID-19 designated hospital: The combination of phylogenetic analysis and field epidemiology study suggesting airborne transmission

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KEYWORDS
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Abstract *Background:* Healthcare-associated COVID-19 infections caused by SARS-CoV-2 have increased morbidity and mortality. Hospitals and skilled nursing facilities (SNFs) have been challenged by infection control and management.
Methods: This case study presents an outbreak investigation in a COVID-19-designated hospital and a hospital-based SNF. Real-time polymerase chain reaction (PCR) and other studies were

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Phylogenetic analysis;
Sulfur hexafluoride;
Airborne transmission

performed on samples obtained from SNF residents, hospital patients, and healthcare workers (HCWs). The results of the laboratory tests and field epidemiological data were analyzed. Genome sequencing and phylogenetic analysis of SARS-CoV-2 were performed to identify the associations between cases. The tracer gas was released and recorded by a thermal imaging camera to investigate the spatial relations within clusters.

Results: During the outbreak, 29 COVID-19 infections in 3 clusters were identified through hospital-wide, risk-guided, and symptom-driven PCR tests. This included 12 HCWs, 5 patients, and 12 SNF residents who had been hospitalized for at least 14 days. Serology tests did not identify any cases among the PCR-negative individuals. The phylogenetic analysis revealed that viral strains from the 3 clusters shared a common mutation of G3994T and were phylogenetically related, which suggested that this outbreak had a common source rather than multiple introductions from the community. Linked cases exhibited vertical spatial distribution, and the sulfur hexafluoride release test confirmed a potential airborne transmission.

Conclusions: This report addressed the advantage of a multi-disciplinary team in outbreak investigation. Identifying an airborne transmission within an outbreak highlighted the importance of regular maintenance of ventilation systems.

Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in 615 million cases and 6 million deaths worldwide, as of September 2022.[1] Although SARS-CoV-2 is genetically closely related to SARS-CoV, the genomic differences have led to different disease patterns,[2] such as the rate of transmission,[3,4] presymptomatic transmission, and the higher viral load in the early phase of the diseases,[5] which make infection control and public health measures are more challenging. COVID-19 poses the greatest threat and higher mortalities to the elderly and those with comorbidities[6–8] and patients who stayed in a crowded facility have higher risks of contracting COVID-19.[8,9] The COVID-19 fatality rate is 11%–34% for residents of skilled nursing facilities (SNFs)[10–12] and 11%–20% for hospitalized patients.[13–16] Therefore, during surges in the epidemic, SNFs and hospitals are vulnerable to COVID-19 outbreaks. Quickly identifying and controlling outbreaks in these densely populated facilities is crucial.

This report summarizes the process and the findings of an outbreak investigation involving 29 healthcare-associated COVID-19 infections from May 25 to July 20 in 2021, when the background prevalence of COVID-19 in Taiwan was less than 500 cases per million population and the infections in healthcare facilities were uncommon.[17] The study demonstrates the use of field epidemiology and phylogenetic analysis to describe the exact size of an outbreak and generates a hypothesis of airborne transmission, which is verified by ancillary studies. This report also informs policymakers and infection prevention and control practitioners on how to improve hospital planning and maintenance in healthcare facilities that provide services for COVID-19.

Methods

Setting and participants

Hospital A is a COVID-19-designated hospital with a catchment population of 500,000. The seven-story building has one intensive care unit (ICU), one respiratory care ward, and seven general wards, including two wards designated for COVID-19. The total number of beds is 424, including 38 COVID-19 single-bedded rooms designated for patients with COVID-19. Only five of the designated rooms are negative pressure isolation rooms. A hospital-based SNF with 59 beds is located in the same building (Fig. 1). Hospital A has 377 staff, including healthcare workers (HCWs), administrators, patient service assistants, porters, and janitors.

Outbreak investigation and infection prevention control (IPC)

On June 8, 2021, a 60-year-old male SNF resident developed a fever, and his nasopharyngeal sample was tested positive for SARS-CoV-2 using a real-time polymerase chain reaction (RT-PCR) test on June 9, 2021. Nine additional infections were identified by testing those who came into close contact with the index case. One patient had symptoms for more than seven days, which showed that there was a potential active transmission in the SNF.

The response to the outbreak was conducted according to the guidance of the Taiwan CDC. The case definition in the outbreak was an individual who was diagnosed with COVID-19 14 days after entering the hospital using a RT-PCR.[18] The IPC measurements are shown in Fig. 2a and Table 1.

The date on which the index patient was identified was defined as Day 0 on June 8, 2021. The outbreak period was defined to have started 14 days prior (May 25) to Day 0 and

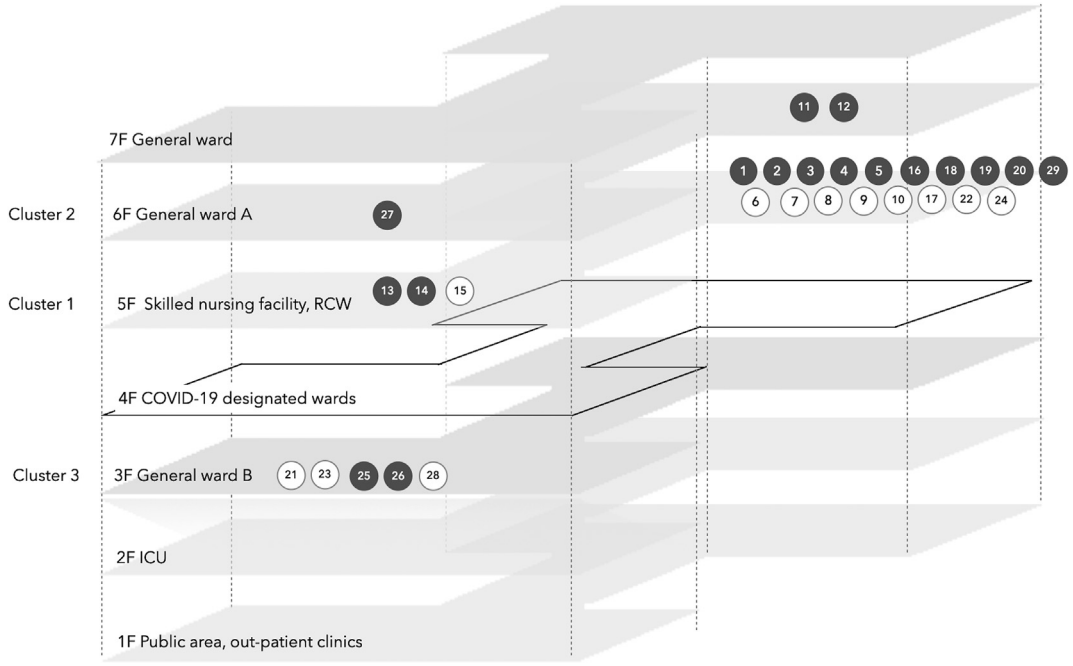

Figure 1. Hospital setting and location for the health care-associated COVID-19 cases. F, floor; ICU, intensive care unit; RCW, respiratory care ward. 1. Numbers in dark grey circles represent COVID-19 cases who are patients or residents; numbers in white circles represent COVID-19 cases who are healthcare workers or staff.

to last for 21 days after any possible environmental or interpersonal exposure to confirmed COVID-19 cases (July 20). Details of contact tracing and patients' clinical information were collected through interviews, medical records, working diaries, and self-reported itineraries. The immediate investigations also involved testing using RT-PCR and serology testing. After the outbreak ended, the process and the findings of the outbreak management study were recorded retrospectively. The phylogenetic analysis of the identified SARS-CoV-2 and a tracer gas release test were conducted. This study was reviewed and approved by the Research Ethics Committee (110-110-E, 110-101-F, and 110-116-E) of National Taiwan University Hospital, Hsin-Chu Branch.

RT-PCR testing strategies

SARS-CoV-2 RT-PCR testing was performed using 3 strategies (Supplementary Table S1): (1) hospital-wide surveillance of all patients from May 14 to June 2 (Day −25 to Day −6), which was extended to all patients, residents, and staff on Jun 11 (Day +3) and July 13 (Day +35); (2) symptom-driven surveillance of all individuals in Hospital A who developed symptoms that were associated with COVID-19 throughout the outbreak period, and (3) risk-guided surveillance (high-risk, intermediate-risk, low-risk individuals).

High-risk individuals were defined as individuals who had contact with a confirmed case of COVID-19 for longer than 15 min with a distance of less than 2 m. For quarantined high-risk individuals, nasopharyngeal sampling was performed on the 1st day and the 14th day after risk identification. However, due to staff shortage, some high-risk individuals were not quarantined; these patients were subjected to aggressive sampling schedules on the 1st day, every 3 days, and on the 14th day after risk identification.

Intermediate-risk individuals were those who stayed or worked on the same ward but had no close contact with any known cases. These individuals were tested weekly. No additional sampling was required for low-risk individuals who did not meet these criteria unless symptom-driven or hospital-wide surveillance demanded sampling.

Serological testing and history of vaccination

Serological testing was used for intermediate- and high-risk individuals on July 27, 2021 (Day +49). Immunoassays for anti-nucleocapsid antibody and anti-spike protein antibody were performed (Elecsys Anti-SARS-CoV-2 S immunoassay, Roche Diagnostics International, Switzerland) and the COVID-19 vaccination history was recorded. Before the outbreak, the coverages of the first dose of the Oxford/AstraZeneca vaccine were 81.2% and 74.4% among healthcare workers and paramedical persons, respectively.

Whole-genome sequencing and phylogenetic analysis

All SARS-CoV-2 strains that were isolated from Hospital A during the outbreak were subjected to whole-genome sequencing and phylogenetic analysis. RNA was extracted from the clinical samples and verified by a positive result for a RT-PCR. Sequencing was performed according to the instruction manual for the Illumina COVID-seq.[19] The steps for COVID-seq testing are summarized in Supplementary Table S2.

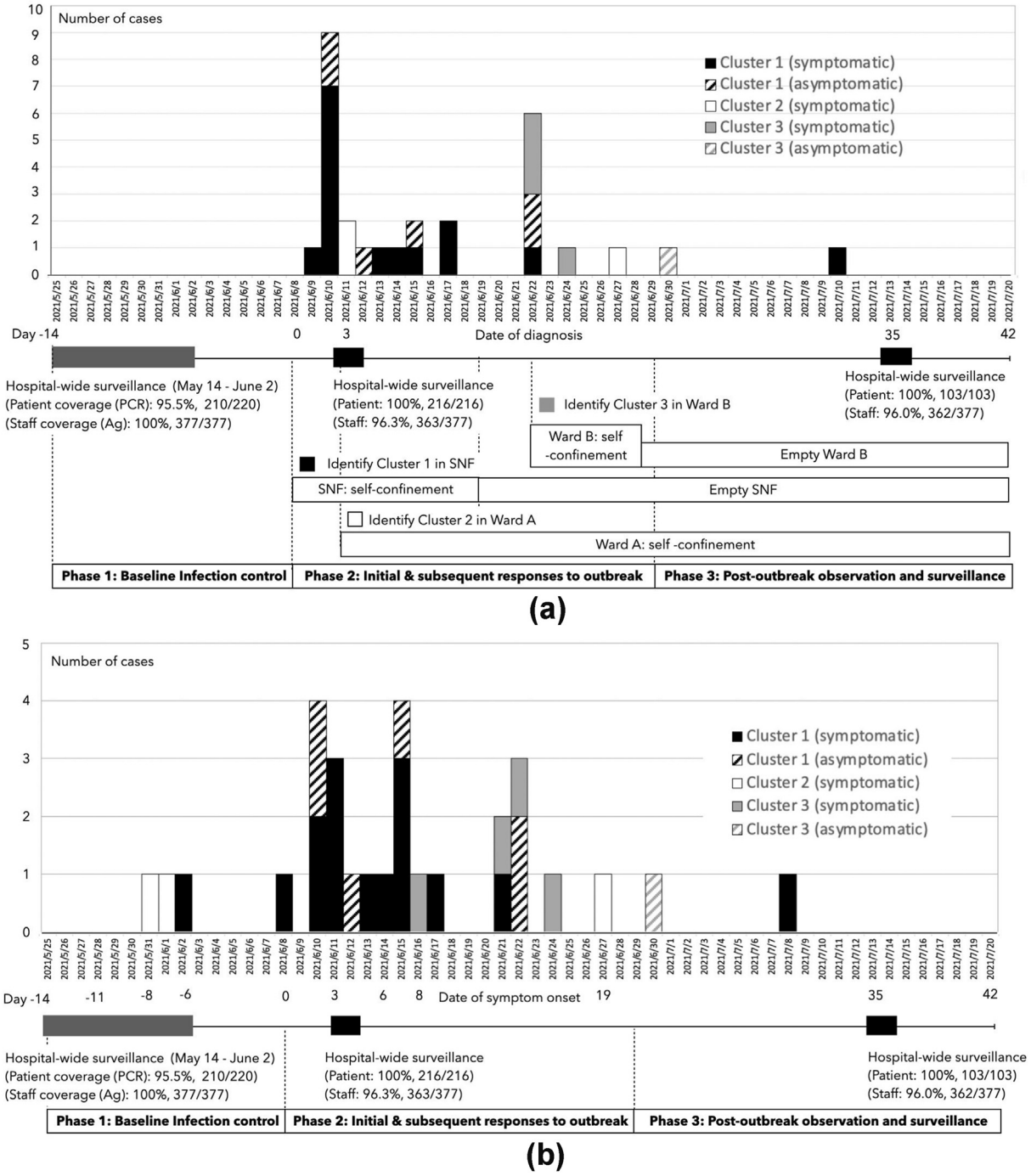

Figure 2. **a**. Epidemic curve according to the date of diagnosis and infection control management. Ag, antigen tests; PCR, polymerase chain reaction; SNF, skilled nursing facility. **b**. Epidemic curve according to the date of the onset of symptoms. Ag, antigen tests; PCR, polymerase chain reaction; SNF, skilled nursing facility.

Whole genomes were collected by initially mapping raw reads to the reference genome of SARS-CoV-2 (accession number: NC_045512.2) using Burrows-Wheeler Aligner (BWA, version 0.7.17).[20] The mapped results were sorted and indexed using Samtools (version 1.7).[21] Using iVar (version 1.0)[22] to trim amplicon primer sequences and generate the consensus sequences with a minimum quality score of 20. Forty-five samples with nearly complete coverage (>98%) showed an average depth of greater than 2200X and these were used for downstream analysis. These sequences were deposited in the Global Initiative on Sharing All Influenza Data (GISAID)[23] with accession numbers provided (Supplementary Table S3).

Visual inspection of mapping results was performed using an Integrative Genomics Viewer (IGV, version 2.11.4)[24] to confirm genomic substitutions and deletions. Two references and all Taiwanese genomes were retrieved from GISAID as of December 2021. An acknowledgments

Table 1 Summary of the outbreak management and the infection control policy and practice.

Setting	Hospital A is a COVID-19-designated hospital with a catchment population of 500,000, which contained 1 ICU, 1 RCW, and 7 general wards, including 2 COVID-19 -designated wards. The total number of beds is 424, including 38 COVID-19-designated beds. Only five of the designated beds are in airborne infection isolation rooms. A hospital based SNF with a capacity of 59 beds is in the same building. There is one full-time equivalent infection control doctor and 2 full-time equivalent infection control nurses.		
Dates	May 25, 2021—July 20, 2021.		
Population characteristic	The total number of hospital patients and residents in the skilled nursing facilities was 220 during the defined outbreak period; the total number of staff during that period was 377. A case is defined as an individual who is diagnosed with COVID-19 using a RT-PCR 14 days after entering the hospital. 29 COVID-19 infections with a mean age of 65.3 were identified; the median length of stay was 17 (interquartile range, 12—26) days.		
Major infection control changes during the study			
Start Date	Stop Date	Infection control practice	Applied site
Phase 1:			
Baseline infection control measurements (May 25, 2021 — June 8, 2021)			
Two weeks prior to the date of index case identification			
February 2020	Until the date of manuscript preparation	**Mask mandate and hand hygiene** at all times	Whole hospital, SNF
		Partitions in meeting rooms and dining areas	Whole hospital
		Self-health monitoring, immediate syndromic surveillance	Whole hospital, SNF
		PPE: N95 respirator, waterproof gowns and gloves	COVID-19 designated wards
		Staff segregation and strict access control: HCWs were not allowed to enter other units	COVID-19 designated wards, SNF
May 14, 2021	June 2, 2021	**Hospital-wide surveillance** of COVID-19 with PCR tests (patients) or antigen tests (staff)	Whole hospital
May 20, 2021	Until the date of manuscript preparation	**Entry restriction**: visitors and contractors were not allowed to enter, all preauthorized visitors were registered, no patients or caregivers were allowed to leave the hospital during their stay	Whole hospital and SNF
		Group rehabilitation activities ceased	Whole hospital
Phase 2			
Initial and subsequent responses to outbreak (June 8, 2021 — June 28, 2021):			
From identification of the first case to complete removal or quarantine of all infected or exposed individuals			
June 8, 2021	June 19, 2021	**Self-confinement:** all patients and patient service assistants quarantined in their rooms	SNF
		PPE for all HCWs involved in daily care: use N95 respirator, waterproof gowns, and gloves at all times in the unit	SNF
		Staff segregation: HCWs did not go to other units	Whole hospital
June 10, 2021	July 20, 2021	**Service load reduction: new admissions ceased, elective surgeries/exams were postponed**	Whole hospital
June 11, 2021	June 12, 2021	**Hospital-wide surveillance** of COVID-19 with PCR tests (patients and staff)	Whole hospital
June 11, 2021	July 20, 2021	**Self-confinement:** all patients quarantined in their rooms; patient service assistant was not allowed	Ward A
		PPE for all HCWs in daily care: use N95 respirator, waterproof gowns and gloves at all times in the unit	Ward A
June 19, 2021	July 20, 2021	**Unit emptying, removal of all residents to other facility**	SNF
June 22, 2021	June 28, 2021	**Self-confinement:** all patients quarantined in their rooms; patient service assistant was not allowed	Ward B
		PPE for all HCWs in daily care: use N95 respirator, waterproof gowns and gloves at all times in the unite	Ward B
June 28, 2021	July 20, 2021	**Unit emptying, removal of all patients to other facility**	Ward B
Phase 3:			
Post-outbreak observation and surveillance (June 29 — July 20)			
3 weeks after the date of complete removal or quarantine of all infected or exposed individuals			
July 13, 2021	July 14, 2021	**Hospital-wide surveillance** of COVID-19 with PCR tests (patients and staff)	Whole hospital

Abbreviation: COVID-19, coronavirus disease 2019; HCW, healthcare worker; ICU, intensive care unit; IQR, inter-quartile range; n, number; PCR, polymerase chain reaction; PPE, personal protective equipment; RCW, respiratory care ward; SD, standard deviation; SNF, skilled nursing facility.

table showing the originating and submitting laboratories for the GISAID sequence data is shown in Supplementary Table S4. A total of 300 whole genomes were used for the phylogenetic analysis. A phylogenetic tree was constructed and visualized using Nextstrain analysis (https://github.com/nextstrain/ncov)[25] and Auspice (https://auspice.us/).

Release and detection of the tracer gas

To determine the spatial relationship between cases, a tracer gas release study was performed using sulfur hexafluoride (SF_6), which was used by previous studies to simulate the ventilation and movement of infectious aerosols.[26,27] The bathrooms of single-bedded rooms without negative pressure were tested. The tracer gas was released into the exhaust fans of the bathrooms (Fig. 5a). The presence and flow of the SF_6 in the affected bathrooms, which were located directly above the bathroom in which gas was released, were recorded using an infrared ray camera (FLIR GF306 optical gas imaging camera, FLIT Advanced Thermal Solutions Inc., France), for which the protocol is shown in Supplementary Tables S5 and S6.

Results

Description of cases

During the investigation, 29 healthcare-associated COVID-19 cases were identified from 3249 clinical samples: 12 staff members and 17 patients/residents. Baseline characteristics, history of vaccination, and outcomes are shown in Table 2. Most cases were identified using symptom-driven and risk-guided surveillance. Only 2 cases (6.9%) were identified via hospital-wide surveillance. The positivity rate was only 0.2% (2/1254) for hospital-wide surveillance, which was less than that for symptom-driven or risk-guided surveillance (1.4%, 27/1995). 37.9% of cases were asymptomatic or pre-symptomatic. The median duration for hospitalization was 17 days. The ICU admission rate and case-fatality rate were both 23.5% (4/29). Due to the limitations of time and human resources during the outbreak, only a limited number of environmental samples (n = 85) were collected on the three floors on which the outbreak occurred and all of these gave a negative result for a RT-PCR (Supplementary Table S3).

Table 2 Summary of all COVID-19 cases for the outbreak.

	All (n = 29)	Patients/residents (n = 17)	Staff (n = 12)	
Baseline characteristics				
Age, years, mean (SD)	65.3 (18.1)	76 (12.7)	49.5 (11.5)	<0.001
Male patients, n (%)	12 (41.4)	11 (64.7)	1 (8.3)	<0.001
Co-morbidities, n (%)	19 (65.5)	17 (100)	2 (16.7)	<0.001
Cardiovascular disease	8 (27.6)	7 (41.1)	1 (8.3)	
Hypertension	13 (44.8)	12 (70.6)	1 (8.3)	
Diabetes mellitus	8 (27.6)	7 (41.1)	1 (8.3)	
Chronic kidney disease	6 (20.7)	6 (35.3)	0	
Malignancy	3 (10.3)	3 (17.6)	0	
Neuropsychiatric disease	8 (27.6)	7 (41.1)	1 (83.3)	
Prior COVID-19 vaccination				
Dosage of vaccination 1 dose, n (%)	10 (34.5)[a]	2 (11.8)[a]	8 (66.7)[a]	0.005
Diagnosis and presentation				
Reason for diagnosis, n (%)				
Symptom-driven	14 (48.3)	10 (58.8)	4 (33.3)	0.109
Hospital-wide surveillance	2 (6.9)	2 (11.8)	0	
Risk-guided	13 (44.8)	5 (29.4)	8 (66.7)	
Pre-symptomatic or asymptomatic, n (%)	11 (37.9)	4 (23.5)	7 (58.3)	0.119
Requiring intensive care, n (%)	4 (13.8)	4 (23.5)	0	0.121
Treatment, n (%)				
Remdesivir	13 (44.8)	9 (52.9)	4 (33.3)	0.451
Steroid	11 (37.9)	8 (47.1)	3 (0.25)	0.273
Tocilizumab	6 (20.7)	5 (29.4)	1 (8.3)	0.354
Monoclonal antibody	4 (13.8)	4 (23.5)	0	0.121
Outcomes				
Duration of hospitalization, days, median (IQR)	17 (12—26)	22 (12—36)	15 (6—22)	0.062
In-hospital mortality, n (%)	4 (13.8)	4 (23.5)	0	0.121

[a] Only 10 cases had just received one dose of Oxford/AstraZeneca COVID-19 vaccine vaccination in less than 1 month prior to their diagnosis of COVID-19 infection. None had received more than one dose. The median time from vaccination to diagnosis was 11 days (range 7—24 days).

Abbreviation: ICU, intensive care unit; IQR, inter-quartile range; n, number; SD, standard deviation.

Epidemic curve and IPC management

The outbreak management and IPC are shown in Table 1 and Fig. 2a. The study identified three clusters: at the SNF, in Wards A and Ward B (Fig. 1). The epidemic curves in Fig. 2a and b respectively show the date of diagnosis and the date for the onset of symptoms. The date on which each case presented to the ward and the date for the onset of symptoms, diagnosis, and mortality are shown in Supplementary Figure S1.

There is no epidemiologic association between Cluster 1 and Cluster 2 because the staff in the two units were isolated from each other. The SNF and Ward A entrance featured access controls that required identification badges. Some janitors worked in these parts of the hospital; but the contact of janitors and patients or residents was limited. Patients in Ward A were quarantined in their rooms but some SNF residents and patients in Ward B were quarantined in rooms with multiple beds because capacity was limited. From the beginning of the study until the end of the outbreak, all staff used personal protective equipment (PPE) and were confined to the facility.

In terms of the beginning of the outbreak in cluster 1, the first case presented with illness on June 2 (Day −6). The study also identified one patient in cluster 3 who had been a resident of the SNF (in the location of cluster 1) until May 28 (Day −11), after which he was transferred to Ward B because of a gastrointestinal bleeding. This patient had a negative RT-PCR result on June 11 (Day +3) and June 14 (Day +6) but developed a fever on June 16 (Day +8). The chain of transmission in the SNF might have presented before Day −11, which also explained the connection between cluster 1 and cluster 3.

The first two cases (Cases 11 & 12) in cluster 2 (Ward A) experienced illness (Day −8 and Day −7, May 30 and June 1) and had been isolated in single-bedded rooms for 1 week prior to the date of diagnosis (Day +3, June 11) with high cycle-threshold values of 27 and 31. All patients in Ward A were quarantined in their rooms from June 11. However, the third case (Case 27) in cluster 2 (Ward A) developed symptoms on June 27 (Day +19), which was 16 days after the last possible direct exposure to the first two cases. The time gap between the three cases shows that there was a longer incubation period or a transmission method other than direct exposure, such as airborne transmission.

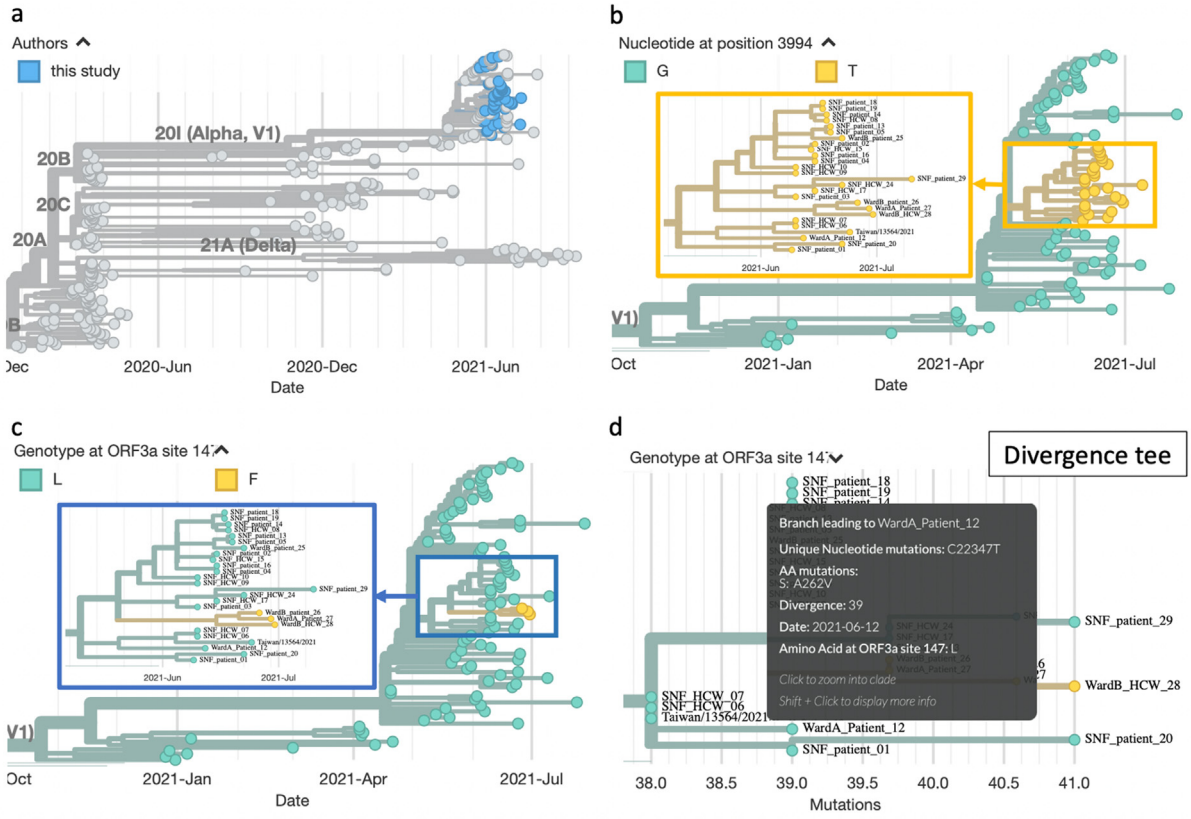

Figure 3. Phylogenetic analysis of SARS-CoV-2 strains from Hospital A in Taiwan. **a**. All strains from Hospital A belong to clade 20I (alpha, V1) in a time-scale tree that is constructed using Nextstrain tool (3a). **b**. Strains from 3 epidemiological clusters in the outbreak are phylogenetically related and share the common mutation, G3994T (3b). **c & d**. Phylogenetically linked cases connect different clusters (3c and 3d). There is a shared amino acid mutation, L147F in ORF3a, for Case 27 in cluster 2 and Cases 26 and 28 in Cluster 3, shown as yellow color in 3c. Cases 6 and 7 in Cluster 1 and Case 12 in Cluster 2 feature only one amino acid change of A262V in Spike, as shown in the divergence tree in Fig. 3d. HCW, healthcare worker; SNF, skilled nursing facility; ORF, open reading frame.

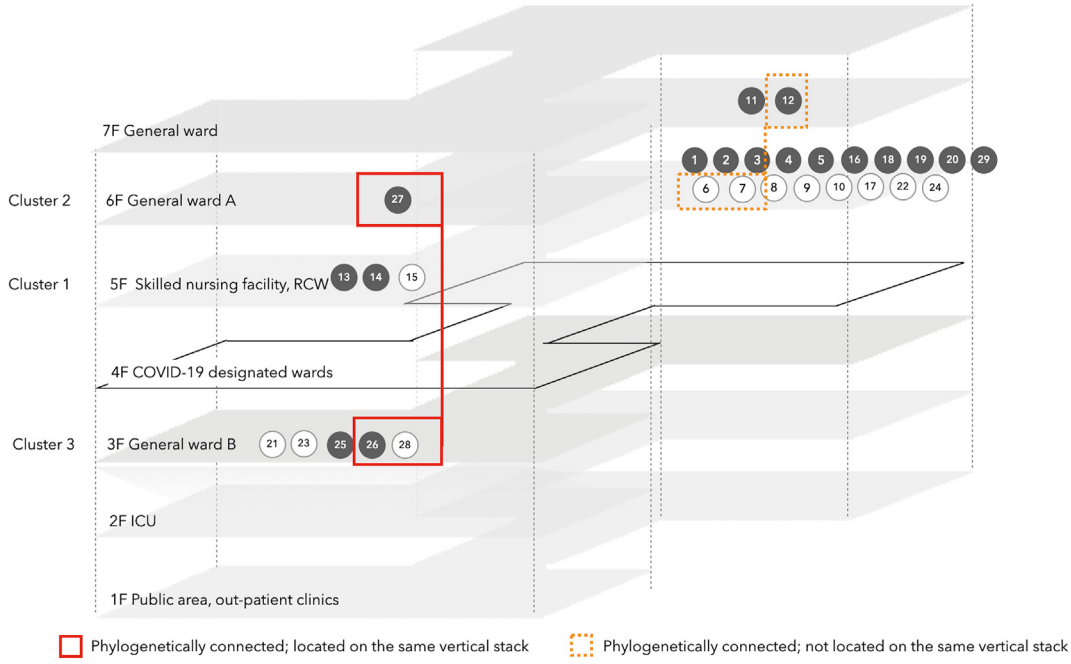

Figure 4. Spatial association between linked cases across outbreak clusters. F, floor; ICU, intensive care unit; RCW, respiratory care ward. 1. Solid lines: Linked cases share a phylogenetic linkage (L147F in ORF3a) and a spatial association. These are located on the same vertical stack. Dashed lines: Linked cases share identical phylogenetic signature, except r only one nucleotide changes.

Serological tests

Serology studies were conducted for 114 patients and residents and 82 HCWs. The serology tests did not identify any additional COVID-19 cases. Test of patients with COVID-19 infections showed positive anti-nucleocapsid protein antibodies and anti-spike antibodies (100%; Supplementary Table S8). The titers were similar for all individuals.

Phylogenetic analysis and the association between linked cases

During the study period, 64 SARS-CoV-2 samples were selected for sequencing, and 45 were used for downstream analysis after quality control. All strains belong to clade 20I (alpha, V1, Fig. 3a). Twenty-five of the 45 samples (55.6%) were from patients who were identified during the outbreak and 20 (44.4%) were from unrelated patients who transferred to the designated hospital after receiving a diagnosis of COVID-19. Strains from 3 clusters in the outbreak were phylogenetically related and shared the common nucleotide mutation, G3994T (Fig. 3b); they were distinct from the strains from other patients who transferred to Hospital A and from all publicly available sequences in Taiwan.

Phylogenetically linked cases connected different clusters (Fig. 3c and d). In Fig. 3d, a shared amino acid mutation from Leu (L) to Phe (F) at position 147 of ORF3a was present in Case 27 in Cluster 2 and in Cases 26/28 in Cluster 3 and Cases 6 and 7 in Cluster 1 and Case 12 in Cluster 2 featured only one amino acid change from Ala (A) to Val (V) at position 262 of Spike. The epidemiological and phylogenetic data showed that there was a vertical spatial distribution between the phylogenetically linked cases (Fig. 4). The patient who was identified as Case 27 in Cluster 2 was located in the same place as those who were identified as Cases 26 and 28 in Cluster 3, on the sixth and third floors, respectively.

Exhaust fan airflow in bathrooms

Each ward has an independent air-cooling system and the air is delivered into each single-bedded room through air-conditioners with an exchange rate of 1.7 times per hour. In an en-suite bathroom of a single-bedded room, the air is extracted by an electrical exhaust fan into a vertical vent stack, which is connected to the exhaust for another bathroom on the same floor and other rooms that are vertically above and below in the same building (Fig. 5a).

The tracer gas release test was conducted for 2 vertical stacks, where cases in Cluster 2 were located. In the first stack, tracer gas was released from the exhaust fans on the fourth floor and was measured at the exhaust fans on the fifth floor, the sixth floor, and the rooftop (Fig. 5b, Supplementary Table S5). The duration of the outflow was longer for a room with a malfunctioning exhaust fan (7 min) than for a room with a properly functioning fan (2 min; Fig. 6a and b). The timing, temperature, and humidity were listed in Supplementary Tables S5 and S6. However, the results were not the same for the second vertical vent stack. The SF_6 release study showed that the airflow travels through the vertical vent stack, but the timing and duration were affected by location and other external factors.

Discussion

To prevent the transmission of COVID-19 infection in facilities, bundles of IPC practices should be implemented, including identifying cases, triaging patients, educating the

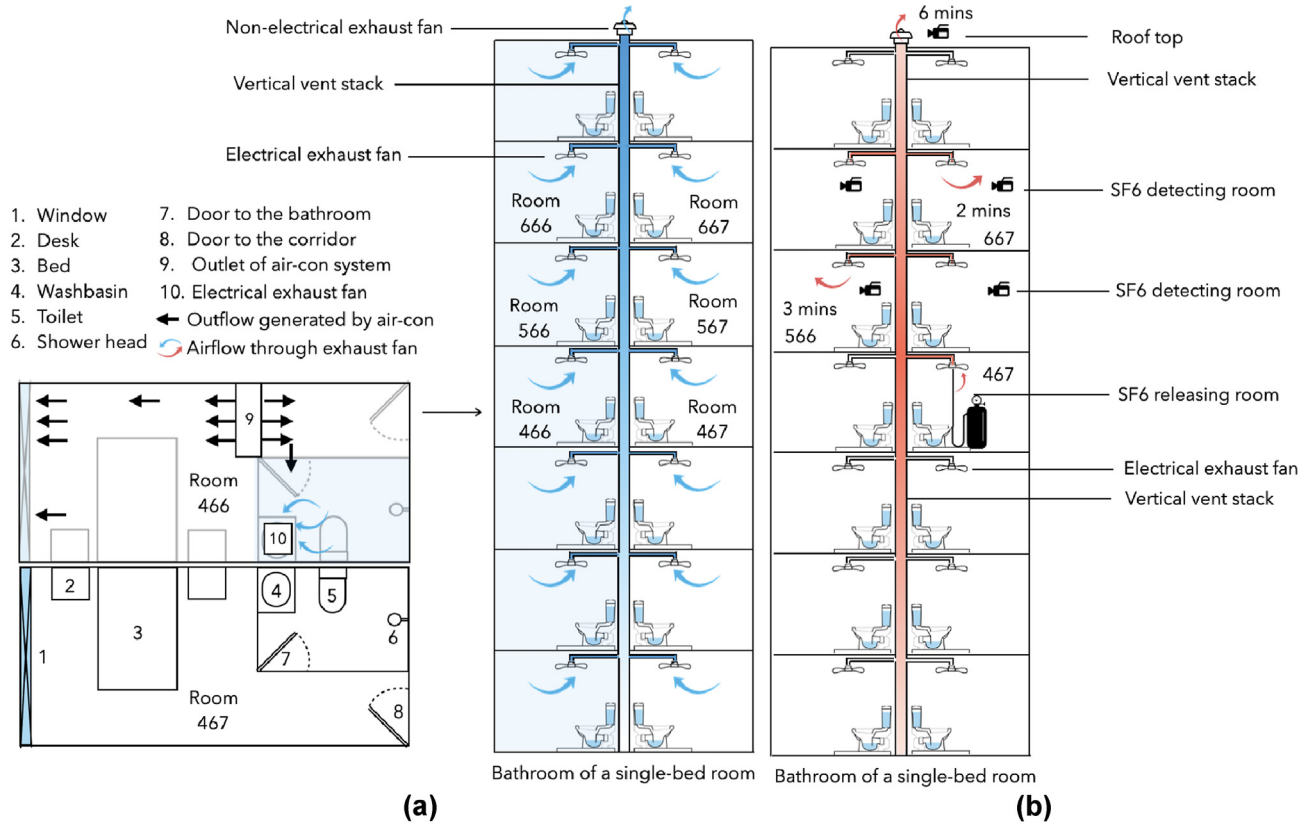

Figure 5. **a**. Illustration of setting and airflow in the ventilation system. **b**. Illustration of sites for the experimental release and detection of the tracer gas, sulfur hexafluoride (SF_6). Bathroom ventilation channels between two adjacent bathrooms and vertically aligned bathrooms are connected.

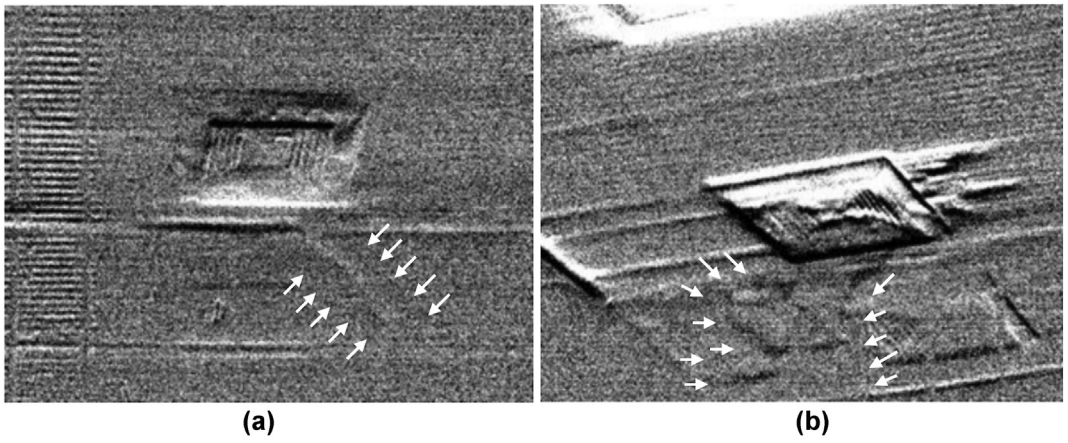

Figure 6. **a**. Detection of the SF_6 outflow. The white arrows show the SF_6 outflow. The outflow of SF_6 was captured from an affected room with an exhaust fan in operation (Room 566), which is located directly above the room where it was released (Room 467). **b**. Greater SF_6 outflow in a room with a malfunctioning exhaust fan. The white arrows show the SF_6 outflow. The outflow of SF_6 is greater for a room with a malfunctioning exhaust fan (Room 667).

use of PPE, and managing exposed workers. For this study, the proportion of asymptomatic infection (37.9%) is less than that in other studies (57%—75%) conducted in SNFs.[12,28] Through symptom-driven and risk-guided surveillance, 1.6% of subjects tested positive, which was higher than 0.2% for hospital-wide surveillance. The results of this study showed that self-health monitoring and risk stratification were effective strategies for limiting the spread of infection.[29] In contrast, serological tests did not identify any additional cases, so the strategy for identifying cases was clinically irrelevant. In terms of triage of patients, patients in Cluster 2 were identified at a late stage of infection with high CT values at the time of diagnosis and stayed in single-bedded rooms, so transmission on Ward A was contained. Custer 1 in the SNF had

the most cases (72%, 21/29) because patients were in two-bedded rooms and patient service assistants stayed in patients' rooms frequently. Our findings agreed with the literature that avoiding crowding in the facilities could mitigate nosocomial outbreaks.[9]

Community exposure to SARS-CoV-2 among HCWs created difficulties for outbreak investigation.[30] Some studies used epidemiological and genomic investigations to guide IPC.[31,32] For this study, whole genomic sequencing and phylogenetic analysis identified a shared mutation (G3994T) in three epidemiological clusters, which distinguished the outbreak cases from those who were transferred to the designated hospital. This finding showed that there was a common source for this outbreak, rather than multiple cases of transmission from the community, and showed that a long-term resident who transferred from the SNF to Ward B caused the subsequent transmission to Ward B.

The SARS-CoV-2 was transmitted by the inhalation of respiratory droplets carrying infectious viruses and the deposition of fluid containing the virus onto mucous membranes. Due to the strict access control for HCW, no evidence of interpersonal transmission was identified in Ward A, indicating another transmission route for cases in this ward. Furthermore, the environmental samples that were obtained after terminal cleaning were all negative, which reduced the possibility of fomite transmission. In fact, the airborne transmission of SARS-CoV-2 by aerosols has been addressed based on its close relationship to SARS.[33] Studies showed that SARS-CoV-2 viruses were persistent and infectious in aerosol form.[34,35] There was new evidence for long-distance airborne transmission of SARS-CoV-2,[36] and some studies showed that the highest concentrations of SARS-CoV-2 RNA occurred in patient toilets or bathrooms.[37] The leakage of foul gas and air contamination through vertical stacks have been studied during the COVID-19 pandemic.[27,38] This study showed that there was a vertical spatial distribution for 3 phylogenetically identical COVID-19 cases that connected different clusters. The tracer gas study also showed that the airflow travelled through a vertical vent stack and regurgitation lasted longer if an exhaust fan was malfunctioning. This result was in agreement with another study,[36] which showed that sufficient air replacement reduced long-distance transmission.

This study had several limitations. First, a high percentage of individuals in Hospital A were tested with RT-PCR but some staff did not return or receive tests. This missing data compromised case identification. Second, fomite transmission could not be excluded completely but the negative environmental sampling, staff segregation, strict access control, and quarantine policy for the affected ward reduced the possibility. Third, the results of the tracer gas release study were not repeatable at different time points and the pressure and temperature distributions within the ventilation stack were not measured. This study did not examine the wastewater drainage system so the effects of aerosol distribution from ventilation openings could not be compared with fecal aerosols that accumulated in the drain systems. Finally, the aerodynamics of SF_6 may not be representative of the aerodynamic properties of respiratory fluid particles carrying SARS-CoV-2. A study of air-sampling at the outflow from a vent with subsequent viral cultures might better support the hypothesis of airborne transmission.[39,40]

In conclusion, this outbreak investigation addressed the advantage of a multi-disciplinary team in identifying the chain of transmission and the weakness of infection control measures in healthcare facilities. This report also highlighted the importance of regular maintenance of ventilation systems in hospitals in the post-COVID-19 era.

Declaration of competing interest

All authors denied any financial and personal conflict of interest that could inappropriately influence this article.

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Appendix A. Supplementary data

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