Overlapping of independent SARS-CoV-2 nosocomial transmissions in a complex outbreak

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Research Article

Keywords: COVID-19, SARS-CoV-2, nosocomial transmission, genomic epidemiology

Posted Date: March 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-305824/v1

Version of Record: A version of this preprint was published at mSphere on August 25th, 2021. See the published version at https://doi.org/10.1128/mSphere.00389-21.

Abstract

SARS-CoV-2 nosocomial outbreaks in the first COVID-19 wave were likely associated to a shortage of personal protective equipment and scare indications on control measures. Having covered these limitations, updates on current SARS-CoV-2 nosocomial outbreaks are required. We carried out an in-depth analysis of a 27-day nosocomial outbreak in a gastroenterology ward in our hospital, potentially involving 15 patients and three healthcare workers. Patients had stayed in one of three neighbouring rooms in the ward. The severity of the infections in six of the cases and a high fatality rate suggested the possible involvement of a single virulent strain persisting in those rooms. Whole genome sequencing of the strains from 12 patients and one healthcare worker revealed an unexpected complexity. Five different SARS-CoV-2 strains were identified, two infecting a single patient each, ruling out their relationship with the outbreak; the remaining three strains were involved in three independent overlapping limited transmission clusters with three, three, and five cases. Whole genome sequencing was key to understand the complexity of this outbreak.

Introduction

Whole genome sequencing allows assessing the diversity acquired worldwide by SARS-CoV-2 and detect the emergence of variants that spread more successfully (1–3). Similarly, genomic analysis has been applied to evaluate local SARS-CoV-2 spread and better understand its transmission dynamics during an outbreak (4–6). Outbreaks in nosocomial settings are of particular relevance as they may affect vulnerable individuals and health care workers, who themselves may become transmission vectors, and are associated to higher risk of mortality (6–8). In the first pandemic wave, the shortage of personal protective equipment (PPE) coincided with limited guidance on the appropriate control measures regarding nosocomial transmission by SARS-CoV-2, which may explain that most nosocomial outbreaks refer to that first period. In the second wave, the shortage of PPE has been covered and control measures implemented, but diversity of the SARS-CoV-2 circulating strains has increased. Thus, research focused on nosocomial outbreaks remains a priority. The aim of the study was to perform an in-depth analysis of a suspected second-wave nosocomial outbreak using WGS.

Patients And Methods

Clinical data

Retrospective study in a tertiary referral hospital in Madrid (Spain) that included all consecutive patients diagnosed with COVID-19 at admission (September 15 to October 12, 2020) in a non-COVID-19 gastroenterology ward, as well as the health care workers (HCWs) that were in charge of these patients and diagnosed during the study period. Baseline characteristics and clinical and laboratory parameters of the patients at COVID-19 diagnosis and their outcome were obtained from their electronic medical records. Data were analysed with the SPSS 20.0 package (SPSS Inc., Chicago, IL, USA). Numeric

variables are expressed as medians and interquartile ranges (IQRs) and categorical variables as the number of cases and their percentages (%).

Diagnostic RT-PCRs

Viral RNA was extracted and purified from 300 µL of nasopharyngeal exudates with the aid of the KingFisher (Thermo Fisher Scientific, Waltham, Massachusetts) instrument. Next, an RT-PCR was performed, using the TaqPath COVID-19 CE-IVD RT-PCR kit (Thermo Fisher Scientific, USA).

Whole genome sequencing

Eleven µL of RNA were used as template for reverse transcription using Invitrogen SuperScript IV reverse transcriptase (ThermoFisher Scientific, Massachusetts, USA) and random hexamers (ThermoFisher Scientific, Massachusetts, USA). Whole genome amplification of the coronavirus was done with an Artic_nCov-2019_V3 panel of primers (Integrated DNA Technologies, Inc., Coralville, Iowa, USA) (artic.network/ncov-2019) and the Q5 Hot Start DNA polymerase (New England Biolabs, Ipswich, Massachusetts, USA). Libraries were prepared using the Nextera Flex DNA Library Preparation Kit (Illumina Inc, California, USA) following manufacturer´s instructions.

Libraries were quantified with the Quantus™ Fluorometer (Promega, Wisconsin, USA), before being pooled at equimolar concentrations (4 nM). Next, they were sequenced in pools of up to 17 libraries on the Miseq system (Illumina Inc, California, USA) and the MiSeq Reagent Micro kit v2 (2x151pb) or in pools of up to 96 libraries with the MiSeq Reagent (2x201 pb).

FastQ files above the GISAID thresholds were deposited at GISAID (EPI_ISL_654287, EPI_ISL_654285, EPI_ISL_654348, EPI_ISL_654204, EPI_ISL_654345, EPI_ISL_654357, EPI_ISL_654203, EPI_ISL_654176, EPI_ISL_654284, EPI_ISL_654292, EPI_ISL_654286, EPI_ISL_654294, EPI_ISL_654288, EPI_ISL_654351, and EPI_ISL_654349).

An in-house analysis pipeline was applied to analyse the sequencing reads. The pipeline can be accessed at https://github.com/pedroscampoy/covid_multianalysis. Briefly, the pipeline goes through the following steps: 1) removal of human reads with Kraken [https://genomebiology.biomedcentral.com/articles/10.1186/gb-2014-15-3-r46]; 2) pre-processing and quality assessment of fastq files using fastp [https://academic.oup.com/bioinformatics/article/34/17/i884/5093234] v0.20.1 (arguments: –cut tail, –cut-window-size, –cut-mean-quality , -max_len1 ,-max_len2) and fastQC v0.11.9 [Andrews S.; S Bittencourt a, “FastQC: a quality control tool for high throughput sequence data – ScienceOpen,” Babraham Inst., p. http://www.bioinformatics.babraham.ac.uk/projects/, 2010.]; 3) mapping with bwa v0.7.17 [H. Li and R. Durbin, “Fast and accurate short read alignment with Burrows-Wheeler transform,” Bioinformatics, vol. 25, no. 14, pp. 1754–1760, 2009.] and variant calling using IVAR v1.2.3 [https://genomebiology.biomedcentral.com/articles/10.1186/s13059-018-1618-7] using Wuhan-1 sequence (NC_045512.2) as reference; 4) Recalibration of punctual low coverage positions using joint

variant calling. When necessary, informative non-covered positions were analysed by standard Sanger sequencing with the corresponding flanking primers from the ARTIC set.

Results

Description of the Outbreak

Fifteen patients (Table 1) admitted to the gastroenterology ward (non-COVID-19 area) within a 27-day period (September15-October12, 2020) were diagnosed with COVID-19, confirmed by positive SARS-CoV-2 RT-PCR. The majority of the patients were male. Hypertension, diabetes, and dyslipidaemia were the most common comorbidities. Six out of the 15 patients (40%) developed bilateral pneumonia. Lymphopenia (950 mm^3: 400–1300) was observed associated to an elevated inflammatory marker. Forty per cent of the patients (6/15) received systemic corticosteroids and 46.7% required oxygen support. Two patients were admitted to the intensive care unit (ICU) and required invasive mechanical ventilation. COVID-19-related mortality was 26.7% (Table 1). Additionally, positive RT-PCRs results were obtained for three HCWs within the same period. A 50-year old male nursing assistant (morning/night rotating shift), with a medical history of seasonal asthma for which he used inhaled short-acting beta-2-agonist as-needed, with excellent control and no clinical exacerbations, and two female nurses aged 36 and 40 with no relevant medical-surgical history. They developed mild symptoms for few days, with positive RT-PCRs on September 24, October 11th, and antigen test on October 12, respectively, confirmed later by PCR later.

Table 1
Demographics, clinical characteristics, and outcomes of patients at diagnosis of SARS-CoV-2

	Patients **N = 15**
Age, years, median (IQR)	67 (51–79)
Males (%)	12 (80)
Comorbidities n (%)	10 (66.7)
- Hypertension	7 (46.7)
- Diabetes	8 (53.3)
- Dyslipidaemia	5 (33.3)
- Respiratory disease	3 (20)
-- COPD	1 (6.7)
-- Obstructive sleep apnoea	2 (13.3)
-- Interstitial lung disease	1 (6.7)
-- Lung cancer	1 (6.7)
- Ischemic heart disease	6 (40)
- Cancer	4 (26.7)
- Chronic liver disease	2 (13.3)
- HIV	1 (6.7)
- Myelodysplastic syndrome	
Radiologic findings n (%)	6 (40)
- Pneumonia	6 (40)
- Bilateral opacities,	

IQR: interquartile range; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus, CRP: C-reactive protein; ICU: Intensive Care Unit

	Patients N = 15
Laboratory findings median value (IQR)	950 (400-1,300)
- Lymphocyte count, mm^3	182,500 (116,250–316,250)
- Platelet count, mm^3	2,032 (466-2,647)
- Ferritin, mg/dL	553 (282-1,642)
- D- dimer, mg/dL	4.15 (1.9–9.3)
- CRP, mg/dL	
Treatments n (%)	6 (40)
- Systemic corticosteroids	3 (20)
- Remdesivir	1 (6.7)
- Tocilizumab	7 (46.7)
- Oxygen therapy	7 (46.7)
–- Nasal cannula	3 (6.7)
–- High-flow nasal cannula	2 (13.3)
–- Invasive mechanical ventilation	7.5 (3)
- Days on invasive ventilation, median (IQR)	
Clinical outcomes n (%)	2 (13.3)
- ICU admissions	10 (66.7)
- Discharge from hospital	5 (33.3)
- Death	4 (26.7)
- Death related to COVID-19	
IQR: interquartile range; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus, CRP: C-reactive protein; ICU: Intensive Care Unit	

The gastroenterology ward consists of 12 rooms with 30 beds (Fig. 1a) increased to 37 beds at the beginning of the second wave. All newly diagnosed COVID-19 cases occupied, at different moments, one of three (two 3-bed rooms and a 2-bed room; Fig. 1a) of the 12 rooms in the ward.

One hundred and twenty-seven patients were admitted to the non-COVID-19 gastroenterology ward during the study period, from which 38 were at risk. Patients who at any time during their stay in the hospital

shared a room with a SARS-CoV2 positive patient were considered at risk (median number (IQR): 4 days (2.75–8.25)) of being infected. Imaging tests results and/or symptoms compatible to COVID-19 allowed making the diagnosis in 14 patients and three HCWs. The remaining case was diagnosed after a screening was done due to close contact with a COVID-19 case. Following the protocol established by the Hospital, a negative RT-PCR was required to be admitted in a non-COVID ward. Therefore, patients were considered as confirmed or probable nosocomial infections; in five cases, the period between their last negative SARS-CoV-2 RT-PCR and a COVID diagnosis was > 14 days and 3–13 days for the remaining eleven cases.

Whole genome sequencing analysis

Genomic analysis of thirteen cases (12 patients and one HCW) for which sequencing material was available, allowed identifying five different SARS-CoV-2 strains: an unexpected diversity (Figure 3). Two of the strains were found each in a single patient (Strains 4 and 5), ruling out the implication of these cases in the outbreak. The remaining three strains were involved in limited independent transmissions with 3, 3, and 5 cases in each cluster (Clusters 1, 2, and 3; 0-1 SNPs within each cluster; Figure 3). The HCW (Case E), initially thought to have been infected after a household exposure, was associated to Cluster 1.

Strain distribution among the three rooms affected by the outbreak (Rooms 1, 2, and 3) is shown in Figure 1b. One-strain-one room association was not observed, but rather a much more heterogeneous situation. Patients infected with different strains had had sequentially stayed in the rooms (three, three, and two different strains identified respectively for patients in rooms 1, 2 and 3, respectively). In addition, there were times at which patients with different strains shared the same room (Figure 1b). From these data, patient-to-patient transmission within the same room or exposure to contaminated surfaces did not seem to be the only explanation for the nosocomial transmissions.

Discussion

Surveillance of SARS-CoV-2 transmission is particularly relevant in hospital environments where exposed subjects are more vulnerable (9). COVID-19 death rates associated to nosocomial infections have been reported to be high, ranging between 33% (5, 10) and 38% (4). Additionally, nosocomial transmission increases the risk of exposure to HCWs (5–7), who may become transmission vectors (6).

Some large nosocomial outbreaks occurred during the first wave of COVID-19, mainly attributed to a shortage of PEP and lack of clear guidance on prevention and control measures. Efforts have been made to increase our knowledge on the dynamics of SARS-CoV-2 nosocomial transmissions in the first wave (4–7, 11).

WGS has been crucial to clarify that the nature of certain outbreaks may differ from the initial assumptions. A nosocomial outbreak in Ireland, where several simultaneous independent outbreaks were at first suspected to involve up to nine different wards (5), was later confirmed to be a limited number of

transversal outbreaks. The use of WGS at the beginning of the first COVID-19 wave left interpretation uncertainties regarding the outbreaks even after genomic analysis (4, 7). Current use of real-time genomic epidemiology has fully proved its benefits (8), probably due to the higher diversity acquired by the currently circulating SARS-CoV-2 strains in comparison to those in the first wave, and a high potential to rule out relationships.

In the second COVID-19 wave, with secured access to PPE and hospital control measures implemented, understanding SARS-CoV-2 nosocomial transmission remains important. In our study, WGS sheds light on the true complexity of a COVID-19 nosocomial outbreak. Once WGS findings were included, the initial assumption of a single outbreak caused by a likely virulent strain (six patients developed pneumonia and 27% had a fatal outcome), interpreted as caused by patient-to-patient transmission and a potential role of contaminated surfaces, provided a completely different perspective.

WGS revealed that five different strains were introduced in the ward. When the outbreak occurred, the gastroenterology ward received COVID-19-free patients from the pulmonology and internal medicine departments. Our WGS-based findings indicate that the currently applied standard measures, only addressed to reduce transmission once the patients are in the ward, are not enough if they not accompanied by additional controls to prevent the introduction of undiagnosed pre-symptomatic or asymptomatic COVID-19 cases.

The five introduced strains coincided in time and involved patients staying in three neighbouring rooms. Despite this spatial-temporal coincidence, only half of the strains were further transmitted and the rest did not cause any secondary cases in the ward. These findings suggest the likely existence of singular, specific factors responsible for the outbreak rather than a general major systematic control deficiency.

The restriction of the outbreak to three neighbouring rooms, with the sequential turnover of different strains in the cases who had occupied the rooms, minimizes the initially assumed possibility of contaminated surfaces. Although the primary transmission mode of SARS-CoV-2 in hospitals is close contact and exposure to droplets or contaminated fomites, our findings suggest airborne transmission. Air turnover was checked in the ward and Room 1 showed the lowest values of inward airflow. Room 3 was not connected to the general ventilation system to impulse air into the room, instead, a fan-coil had been installed that can be manually switched off (following inspection it was disconnected). Finally, Room 2 was adjacent to the one with the lowest pressure of inward airflow (Room 1), which may cause airflow distortions when doors are kept open. These data suggest that possible deficiencies in the air system may be a contributing factor for the concentration of cases in the three indicated rooms.

This study shows the importance of WGS-based analysis to correctly understand the true complexity behind nosocomial transmission events. This technique provided key data to interpret the herein reported outbreak and the description of a reactivation, which was subsequently responsible for one of the three overlapping outbreaks. Moreover, the involvement in the outbreak of a HCW, initially thought to have acquired the infection outside the hospital, was only understood once WGS data were available.

In summary, we report a complex epidemiological scenario of a nosocomial COVID-19 outbreak in the second wave based on WGS. Initially, standard epidemiological findings led to assume a homogeneous outbreak caused by a single SARS-CoV-2 strain, likely virulent, driven by person-to-person transmission and contaminated surfaces. The discriminatory power of WGS offered a notoriously different perspective consisting of five importations of different strains, with only half of them causing secondary cases in three independent overlapping clusters and with a turnover of strains within the same rooms that ruled out the role of contaminated sources.

Declarations

Acknowledgements

We are grateful to Dainora Jaloveckas (cienciatraducida.com) for editing and proofreading assistance.

Funding

This work was supported by *Instituto de Salud Carlos III* (Ref COV20/00140: SeqCOVID - *Consorcio para la epidemiología genómica de SARS-CoV-2 en España*) and by *Consejo Superior de Investigaciones Científicas* (*CSIC*) (*PTI Salud Global*). LPL holds a Miguel Servet Contract CP15/00075).

Conflict of interests

The authors do not have commercial or other associations that might pose a conflict of interest.

Availability of data and material

WGS data are deposited in GISAID (See Methods)

Code availability

Bioinformatic Pipelines are available (See Methods)

Ethics approval

The study was approved by the ethical research committee of Gregorio Marañón Hospital (REF: MICRO.HGUGM.2020-042)

Consent to participate

Informed consent was obtained

Consent for publication

Consent for publication was obtained

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References

1. Alm E, Broberg EK, Connor T, et al. Geographical and temporal distribution of SARS-CoV-2 clades in the WHO European Region, January to June 2020. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2020;25(32). doi:10.2807/1560-7917.ES.2020.25.32.2001410.
2. Hodcroft EB, Zuber M, Nadeau S, et al. Emergence and spread of a SARS-CoV-2 variant through Europe in the summer of 2020. medRxiv: the preprint server for health sciences. 2020. doi:10.1101/2020.10.25.20219063.
3. Rambaut ALN, Pybus O, Barclay W, Barrett J, Carabelli A, Connor T, Peacock T, Robertson DL, Volz E. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. ARTIC Network: Report; 2020.
4. Lessells R, Moosa Y. oliveira Td. Report into a nosocomial outbreak of coronavirus disease 2019 (COVID-19) at Netcare St Augsutine´s Hospital. 2020.
5. Lucey M, Macori G, Mullane N, et al. Whole-genome sequencing to track SARS-CoV-2 transmission in nosocomial outbreaks. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2020. doi:10.1093/cid/ciaa1433.
6. Wang X, Zhou Q, He Y, et al. Nosocomial outbreak of COVID-19 pneumonia in Wuhan, China. Eur Respir J. 2020;55(6). doi:10.1183/13993003.00544-2020.
7. Cao G, Tang S, Yang D, et al. The Potential Transmission of SARS-CoV-2 from Patients with Negative RT-PCR Swab Tests to Others: Two Related Clusters of COVID-19 Outbreak. Jpn J Infect Dis. 2020;73(6):399–403. doi:10.7883/yoken.JJID.2020.165.
8. Meredith LW, Hamilton WL, Warne B, et al. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. Lancet Infect Dis. 2020;20(11):1263–72. doi:10.1016/S1473-3099(20)30562-4.

9. El-Solh AA, Lawson Y, Carter M, El-Solh DA, Mergenhagen KA. Comparison of in-hospital mortality risk prediction models from COVID-19. PloS one. 2020;15(12):e0244629. doi:10.1371/journal.pone.0244629.
10. Rickman HM, Rampling T, Shaw K, et al. Nosocomial transmission of COVID-19: a retrospective study of 66 hospital-acquired cases in a London teaching hospital. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2020. doi:10.1093/cid/ciaa816.
11. Sikkema RS, Pas SD, Nieuwenhuijse DF, et al. COVID-19 in health-care workers in three hospitals in the south of the Netherlands: a cross-sectional study. Lancet Infect Dis. 2020;20(11):1273–80. doi:10.1016/S1473-3099(20)30527-2.

Figures

Figure 1

Ward room and bed layout (Rooms 1, 2, and 3 in which cases accumulated are numbered). A) In black the distribution of SARS-CoV-2 cases throughout three periods, B) in orange, yellow, pink, blue, green and black, the distribution of cases after having obtained the genomic data to differentiate between the involved strains.

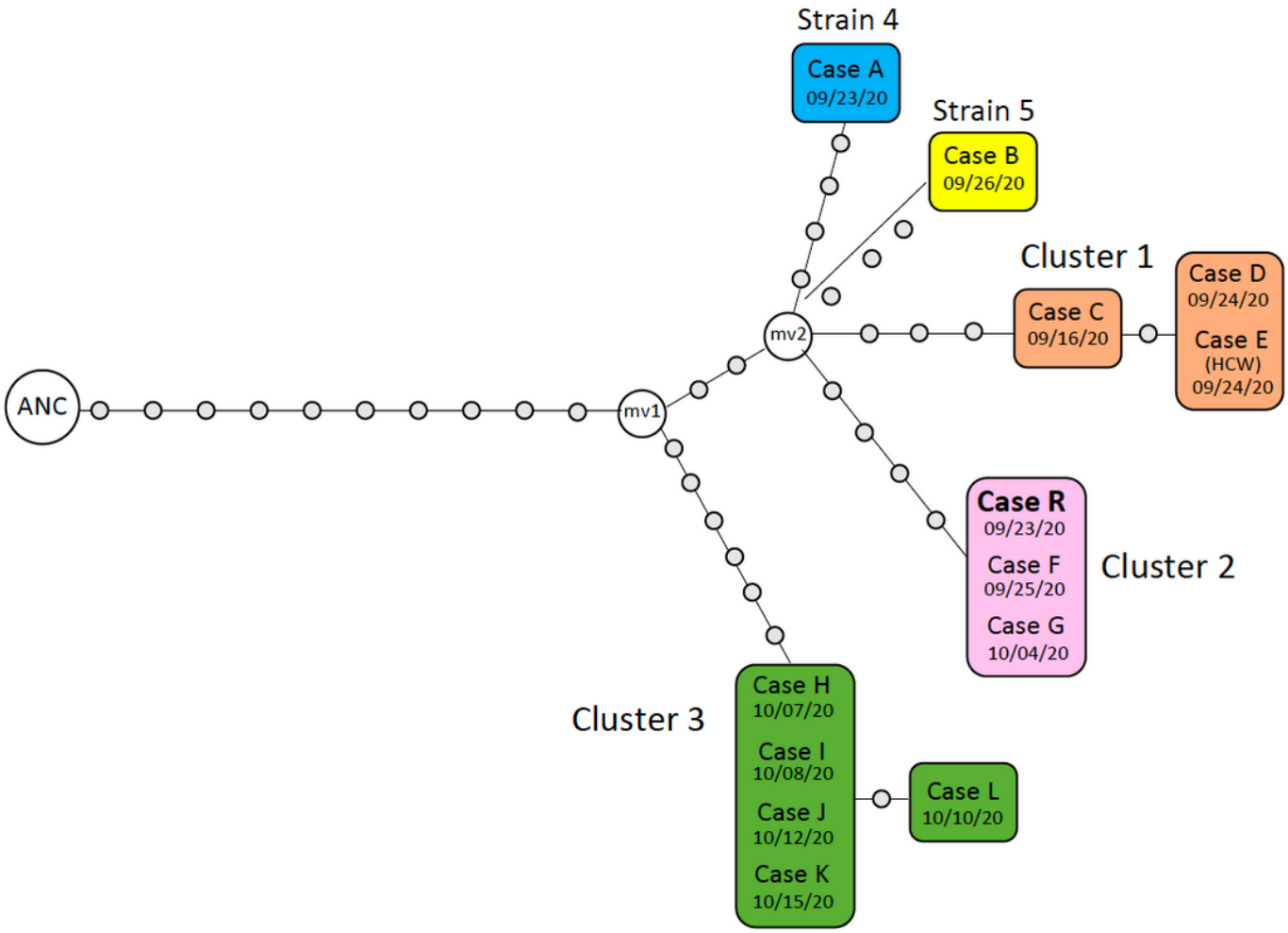

Figure 2

Clinical timeline for Case R. ERCP: endoscopic retrograde cholangiopancreatography; RT-PCR: Reverse-transcription polymerase chain reaction; S: serum sample; NP nasopharyngeal sample; (+) Positive result; (-) Negative result; RBC: red blood cells transfusion. CT: computerized axial tomography scan. MO failure: multiorgan failure; HFNC: high-flow nasal cannulas; O. intubation: orotracheal intubation

Image not available with this version

Figure 3

Network of relationships obtained from whole genome sequencing analysis for the outbreak strains. Each dot corresponds to a single nucleotide polymorphism. When two or more cases share identical genome (zero single nucleotide polymorphisms between them) they are included in the same box. mv: median vector: not sampled recent common ancestor for the two branches. ANC: Wuhan-1 reference strain.