This is an Accepted Manuscript for *Infection Control & Hospital Epidemiology* as part of the Cambridge Coronavirus Collection.

DOI: 10.1017/ice.2022.126

**Title**
The limits of genomic sequencing for SARS-CoV-2 exposure investigations: for nosocomial outbreak reconstruction, community exposures matter too

**Authors**
Liang En, Wee;1 Karrie Kwan-Ki Ko;2,3 Edwin Philip Conceicao;4 May Kyawt Aung;4 Aung Myat Oo;4 Yang Yong;4 Shalvi Arora;4 Indumathi Venkatachalam1,4

**Affiliations**
1. Department of Infectious Diseases, Singapore General Hospital, Singapore
2. Department of Molecular Pathology, Singapore General Hospital
3. Department of Microbiology, Singapore General Hospital
4. Department of Infection Prevention and Epidemiology, Singapore General Hospital, Singapore

**Corresponding Author:**
Dr Wee Liang En Ian, Singapore General Hospital
Email: ian.wee.l.e@singhealth.com.sg
Telephone Number: +65 96777651
ORCID: 0001-6428-9999

**Keywords**
SARS-CoV-2; COVID-19; infection control; nosocomial; healthcare-associated; healthcare workers; delta variant

**Declarations**

**Funding**
This work was not grant-funded. Funding for consumables was provided by the Singapore General Hospital.

**Conflict of interest**
The authors report no conflicts of interest

**Ethics statement**
As this study was conducted as part of outbreak investigation, ethics approval was not required under our institutional review board guidelines.
Dear Editor,

We read with interest the findings of Smith et al.\(^1\) in which genomic sequencing was utilized to clarify transmission-events amongst healthcare-workers (HCWs) and patients. Results of genomic sequencing and epidemiological investigations of in-hospital exposure were combined to identify putative nosocomial transmission-events; however, information on potential exposures of HCWs/patients to COVID-19 in the community was unavailable. Both community and intra-hospital exposures contribute to SARS-CoV-2 acquisition amongst HCWs.\(^2\) Rapid transmission and a relatively slowly evolving pathogen limits the value of sequencing when not augmented with exposure data.\(^3\) In the absence of information on community exposures, relying on genomic sequencing may misclassify coincidental infections of HCWs/patients as an intra-hospital transmission cluster, particularly during periods of low viral diversity, such as during the emergence of a SARS-CoV-2 variant.\(^4\) We would like to share our experience on the limitations of sequencing in investigating nosocomial transmission during initial emergence of the SARS-CoV-2-delta-variant. Despite availability of ample data from sequencing and intra-hospital outbreak investigations, additional epidemiological evidence from community-wide contact tracing was still required to thoroughly evaluate potential nosocomial transmission.

In Singapore, a large nosocomial COVID-19 outbreak attributed to the SARS-CoV-2 delta-variant in April 2021\(^5\) provided impetus for inpatient and HCW surveillance via weekly routine-rostered-PCR-testing at our institution, the largest tertiary-hospital in Singapore (1785-beds).\(^6\) Given that our hospital was located in downtown Singapore, one of the most densely-populated cities, there was risk of spillover from COVID-19 outbreaks in the surrounding community (Figure 1a).\(^6\) Intensive community-surveillance for COVID-19 clusters was conducted by our national Ministry-of-Health, with usage of digital contact-tracing tools made mandatory to register entry/exit to/from areas of high human traffic/enclosed indoor spaces; allowing for retrospective contact-tracing when community COVID-19 clusters were detected. A history of having visited COVID-19 community clusters was thus considered a significant epidemiological risk factor and all inpatients were routinely asked to provide this history on admission triage;\(^7\) similarly, HCWs who were retrospectively identified as having visited these clusters were required to notify our hospital’s epidemiology department.\(^8\)
Information on community exposure (visiting known COVID-19 community clusters) could thus be integrated into outbreak-investigation of nosocomial COVID-19 cases. Our institution reported its first potential nosocomial-onset COVID-19 case in Sept 2021 (defined as PCR-positive ≥7 days(d) from initial admission); soon afterwards a cluster of nosocomial-onset-COVID-19 cases was detected on a renal ward. We utilized contact-tracing and genomic sequencing to investigate nosocomial-onset-COVID-19 cases. All inpatient COVID-19 cases and HCW cases over a 1-month period (20th August-17th September 2021) with a cycle-threshold(CT)-value of <31 were sent for sequencing using the ARTIC protocol on Oxford Nanopore miniION sequencers; contact-tracing was performed for all nosocomial-onset-COVID-19 cases, all community-onset-COVID-19 cases initially managed outside of isolation-areas, as well as all HCWs at work during their infective periods. Epidemiological outbreaks were defined as ≥2 cases of COVID-19 in patients/HCWs with significant close-contact, defined as contact within 2-metres of the index-case for ≥15 minutes, during the index-case’s infectious-period. Genomic clusters were detected based on whole-genome-similarity analysis (when sequences are ≤3 SNPs different and fall in the same branch of the genome-similarity-tree).

On genomic sequencing, the majority of nosocomial-onset/HCW cases clustered on a separate phylogenetic branch from community-onset COVID-19 cases managed in isolation from the onset (Figure 1a), suggesting disparate introductions. However, an identical sequence match was observed between a possible nosocomial-onset-COVID-19 case and an HCW who had both been on the renal ward. Inpatient A was initially admitted to the renal ward for 3d and tested positive upon readmission 4d post-discharge. HCW B tested positive 2d later; HCW B had worked daily on the renal ward 2-weeks prior to diagnosis (during the period of inpatient A’s initial admission), although HCW B did not directly care for inpatient A (Figure 1b). A total of 191 HCWs and 41 inpatients were additionally identified as having had significant close-contact and placed on enhanced-surveillance (d1/d4/d7/d10 PCR-post-exposure); none tested positive subsequently. Based on genomic analysis and intra-hospital outbreak investigation alone, nosocomial transmission could not be ruled out given overlap in space and time. However, when information on community exposures was taken into account, both HCW B and inpatient A had visited community cluster A (inpatient A between discharge and readmission; HCW B, after work) (Figure 1b). Indeed, based on
genomic analysis, inpatient A/HCW B clustered together with other community-onset inpatient cases with reported exposure to community cluster A who were managed in isolation from the onset, and not with other nosocomial-onset-COVID-19/HCW cases (Figure 1a). Sequencing linkage between inpatient A/HCW B more likely reflected acquisition from a common community source, rather than nosocomial transmission; however, this would not have been readily apparent without information on their community exposures.

Despite the potential for genomic sequencing in clarifying nosocomial transmission of SARS-CoV-2, possible pitfalls in interpretation still exist. Our experience highlights that thorough epidemiological investigation, including both intra-hospital and community exposures, remains important in investigating nosocomial COVID-19 outbreaks.
References


Figure 1: Combining genomic sequencing and epidemiological investigation of intra-hospital/community exposure to COVID-19 cases

Initially managed outside of isolation
- Possible nosocomial-onset inpatient case
- Possible nosocomial-onset inpatient case (renal ward exposure)
- HCW

Managed in isolation from onset
- Community-onset inpatient case with exposure to community cluster A
- Imported cases (travellers)

Community-onset inpatient case with exposure to other community clusters

Possible nosocomial-onset inpatient A (renal ward)
HCW B (worked on renal ward)

Community-onset inpatient cases with exposure to community cluster A (managed in isolation from onset, no intra-hospital contact with inpatient A/HCW B)

Nosocomial-onset cluster

Overlapped in ward

Ref: Reference (B)

Incubation periods were defined as 1–14 days prior to positive-PCR.

Infectious period was defined as 2 days prior to symptom-onset if symptomatic or 7 days prior to positive-PCR if asymptomatic; to 10 days after positive-PCR.

Timeline:
- D1: X = negative SARS-CoV-2 PCR test
- D2: X = positive SARS-CoV-2 PCR test
- D3: X = visited community cluster A
- D4: D5: D6: D7: D8: D9:
  - Isolated upon readmission
  - Placed on furlough