The limits of genomic sequencing for severe acute respiratory coronavirus virus 2 (SARS-CoV-2) exposure investigations: For nosocomial outbreak reconstruction, community exposures matter, too

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To the Editor—We read with interest the findings of Smith et al[1] in which genomic sequencing was utilized to clarify transmission events among 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 and patients to coronavirus disease 2019 (COVID-19) in the community was unavailable. Both community and intrahospital exposures contribute to severe acute respiratory coronavirus virus 2 (SARS-CoV-2) acquisition among HCWs.[2] Rapid transmission and a relatively slowly evolving pathogen limits the value of sequencing when it is not augmented with exposure data.[3] In the absence of information on community exposures, relying on genomic sequencing may misclassify coincidental infections of HCWs and/or patients as an intrahospital transmission cluster, particularly during periods of low viral diversity, such as during the emergence of a SARS-CoV-2 variant.[4] Here, we share our experience regarding the limitations of sequencing in investigating nosocomial transmission during initial emergence of the SARS-CoV-2 δ (delta) variant. Despite the availability of ample data from sequencing and intrahospital outbreak investigations, additional epidemiological evidence from communitywide 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 δ (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-care hospital in Singapore (1,785 beds).[6] Given that our hospital is located in downtown Singapore, a densely populated city, there was a risk of spillover from COVID-19 outbreaks in the surrounding community (Fig. 1a).[6] Intensive community surveillance of COVID-19 clusters was conducted by the Singapore Ministry of Health using digital contact-tracing tools made mandatory to register entry or exit to or from areas of high human traffic or enclosed indoor spaces. This surveillance allowed retrospective contact tracing when community COVID-19 clusters were detected. A history of having visited an area with a COVID-19 community cluster was 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 an area with a known COVID-19 community clusters) could thus be integrated into the outbreak investigation of nosocomial COVID-19 cases. Our institution reported its first potential nosocomial-onset COVID-19 case in September 2021 (defined as PCR positive ≥7 days from initial admission). Soon after, a cluster of nosocomial-onset COVID-19 cases was detected on a renal ward.[9] We utilized contact tracing and genomic sequencing to investigate nosocomial-onset COVID-19 cases.[10] All inpatient COVID-19 cases and HCW cases over 1 month (August 20–September 17, 2021) with a cycle threshold value (Ct) <31 were sent for sequencing using the ARTIC protocol on Oxford Nanopore minION sequencers (Oxford Nanopore Technologies, Oxford, UK). Contact tracing was performed for all nosocomial-onset COVID-19 cases, all community-onset COVID-19 cases initially managed outside 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 and HCWs with significant close contact, defined as contact within 2 m of the index case for ≥15 minutes, during the infectious period of the index case. Genomic clusters were detected based on whole-genome similarity analysis (ie, when sequences are ≤3 single-nucleotide polymorphisms different and fall in the same branch of the genome similarity tree).[10]

As revealed by genomic sequencing, most nosocomial-onset and HCW cases clustered on a separate phylogenetic branch from community-onset COVID-19 cases managed in isolation from the onset (Fig. 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 3 days and tested positive upon readmission 4 days after discharge. HCW B tested positive 2 days 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 (Fig. 1b). In total, 191 HCWs and 41 inpatients were additionally identified as having had significant close contact and were placed on enhanced surveillance

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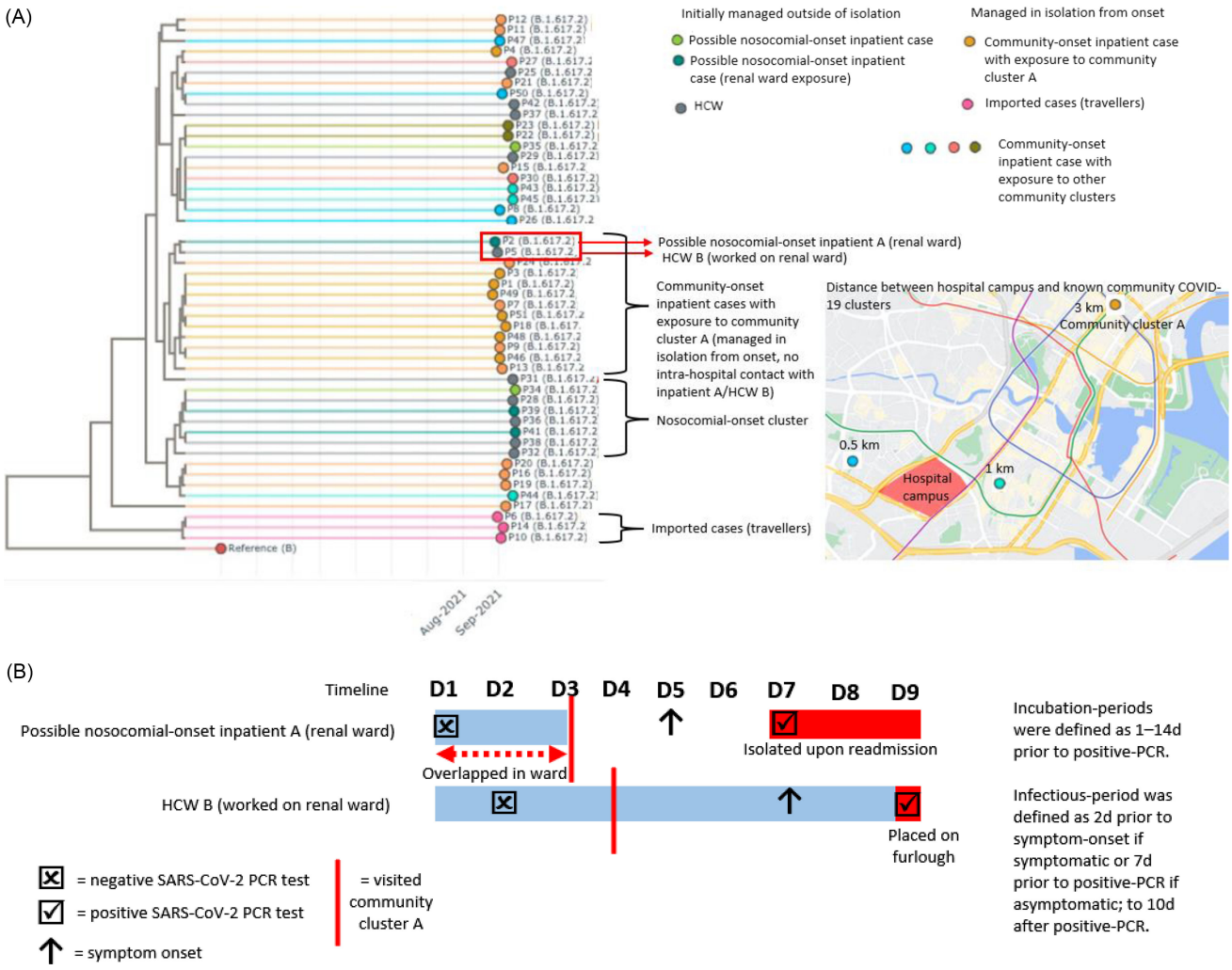

Fig. 1. Combining genomic sequencing and epidemiological investigation of intrahospital and community exposure to COVID-19 cases.

(ie, PCR tests on day 1, day 4, day 7, and day 10 after exposure). None subsequently tested positive. Based on genomic analysis and intrahospital outbreak investigation alone, nosocomial transmission could not be ruled out given overlap in space and time. However, when information on community exposures was considered, both HCW B and inpatient A had visited community cluster A (inpatient A between discharge and readmission; HCW B, after work) (Fig. 1b). Indeed, based on genomic analysis, inpatient A and 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. Inpatient A and HCW B did not cluster with other nosocomial-onset and HCW COVID-19 cases (Fig. 1a). The sequencing linkage between inpatient A and HCW B more likely reflected acquisition from a common community source rather than nosocomial transmission. However, this information 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 intrahospital and community exposures, remains important in investigating nosocomial COVID-19 outbreaks.

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Nosocomial severe acute respiratory coronavirus virus 2 (SARS-CoV-2) transmission arising from a case of N-gene dropout on reverse-transcription polymerase chain reaction (RT-PCR) testing

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To the Editor—During the coronavirus-disease-2019 (COVID-19) pandemic, in-hospital surveillance via serial severe acute respiratory coronavirus virus 2 (SARS-CoV-2) testing has repeatedly demonstrated its utility in early detection, isolation, and interruption of nosocomial transmission.[1] However, analytical sensitivity of real-time, reverse-transcriptase, polymerase chain reaction (rRT-PCR) testing for SARS-CoV-2 is crucial in ensuring accurate results and early detection of COVID-19 cases and, thus, to mitigate nosocomial COVID-19 outbreaks. The emergence of SARS-CoV-2 variants has been demonstrated to negatively affect analytical sensitivity of rRT-PCR assays. For instance, failure of the N-gene assay has occurred with certain SARS-CoV-2 variants.[2] N-gene point mutations have been reported in the literature, resulting in cases of diagnostic escapes.[3–5] To date, however, no confirmed case of nosocomial transmission arising from N-gene dropout has been reported in the literature. We highlight here a case of nosocomial SARS-CoV-2 transmission arising from a case of N-gene dropout, which illustrates the importance of proper interpretation when discrepant results are encountered on different gene-targets utilized in SARS-CoV-2 diagnostic testing. This study was conducted as part of an outbreak investigation, and ethics approval was not required under our institutional review board guidelines.

At our institution, a large tertiary-care hospital in Singapore, from June 21, 2021, onward, all inpatients were routinely tested for SARS-CoV-2 on admission via both rapid antigen detection (RAD) testing as well as PCR, as part of enhanced infection-prevention measures.[6,7] Routine SARS-CoV-2 PCR testing was conducted using the Cepheid GeneXpert Xpert Xpress assay (Cepheid, Sunnyvale, CA), a proprietary FDA-approved assay targeting both the E-gene and N-gene; while the BD-Veritor SARS-CoV-2 antigen rapid test kit (Becton Dickinson, Franklin Lakes, NJ) was used for SARS-CoV-2 RAD testing. Inpatients were subsequently tested at weekly intervals for SARS-CoV-2 infection via PCR.[7] Up to January 2022, 49,933 admissions were screened for SARS-CoV-2, with 3,155 (6.3%) of 49,933 testing positive. In January 2022, an asymptomatic female aged 74 years (patient A), doubly vaccinated with mRNA vaccines, was admitted to our institution. SARS-CoV-2 RAD testing on admission was negative; SARS-CoV-2 PCR on admission screening via GeneXpert-Xpert-Xpress returned a positive result on the E-gene gene target (cycle-threshold [Ct], 20.7) but a negative result on the N2 gene target. Because our institution had not encountered N-gene dropout cases among hospitalized inpatients prior to January 2022, the practice at that time was to await results of repeated SARS-CoV-2 PCR testing using a different assay (Roche cobas-6800, Roche Diagnostics, Indianapolis, IN) that utilized a separate set of gene targets (*ORF-1a* and E-gene regions). As such, the patient was not initially isolated until repreated testing revealed persistent positive results at a low Ct value for the E-gene target and repeated SARS-CoV-2 PCR using a different assay (ie, Roche cobas-6800) returned positive. The index patient was subsequently transferred

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