infection prevention and control measures and robust sickness-surveillance systems? *Infect Control Hosp Epidemiol* 2021. doi: 10.1017/ice.2021.268.
6. Wee LE, Conceicao EP, Aung MK, Oo AM, Yong Y, Venkatachalam I, Sim JX. Rostered routine testing for healthcare workers and universal inpatient screening: the role of expanded hospital surveillance during an outbreak of COVID-19 in the surrounding community. *Infect Control Hosp Epidemiol* 2021. doi: 10.1017/ice.2021.366.
7. Wee LE, Hsieh JYC, Phua GC, *et al.* Respiratory surveillance wards as a strategy to reduce nosocomial transmission of COVID-19 through early detection: the experience of a tertiary-care hospital in Singapore. *Infect Control Hosp Epidemiol* 2020;41:820–825.
8. Wee LE, Sim XYJ, Conceicao EP, *et al.* Containment of COVID-19 cases among healthcare workers: the role of surveillance, early detection, and outbreak management. *Infect Control Hosp Epidemiol* 2020;41:765–771.
9. Wee LE, Conceicao EP, Sim JX, *et al.* Sporadic outbreaks of healthcare-associated COVID-19 infection in a highly vaccinated inpatient population during a community outbreak of the B.1.617.2 variant: the role of enhanced infection-prevention measures. *Am J Infect Control* 2022;50:465–468.
10. Wee LE, Ko KKK, Conceicao EP, *et al.* Linking sporadic hospital clusters during a community surge of the SARS-CoV-2 delta variant (B.1.617.2): the utility of whole-genome sequencing. *Infect Control Hosp Epidemiol* 2022. doi: 10.1017/ice.2022.106.

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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Cite this article: Wee LEI, *et al.* (2023). 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. *Infection Control & Hospital Epidemiology*, 44: 154–156, https://doi.org/10.1017/ice.2022.170

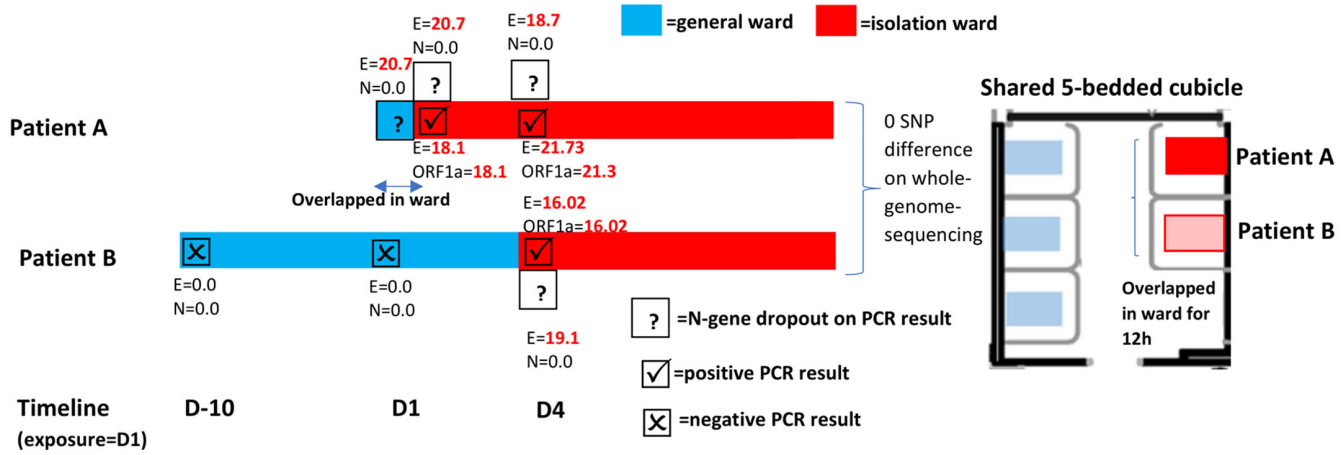

Fig. 1. Nosocomial transmission arising from a case of N-gene diagnostic escape.

to isolation. Also, 5 other patients in the shared cubicle were exposed. The fully vaccinated patient in the adjacent bed (patient B), with an exposure time of 12 hours, subsequently tested positive on day 4 after exposure (ie, day 14 of admission), with a similar pattern of N-gene dropout on testing (Fig. 1). Previously, our institution reported 2 cases of N-gene dropout arising from a point mutation in G29195T; however, these cases were submitted from an outpatient ambulatory clinic in October 2021 and did not overlap with this cluster of cases in space or time.[5]

The nasopharyngeal swab samples of patient A and patient B were subjected to whole-genome sequence analysis. A point mutation, C29200T, was detected in the consensus sequences of both samples. G29200T is a synonymous mutation that was previously reported to cause N-gene detection failure.[4] Whole-genome similarity analysis was performed using previously published workflows utilizing the ARTIC protocol on Oxford Nanopore minION sequencers.[8] The SARS-CoV-2 genomes from patient A and patient B were identical (0 SNP difference). The identical genomes and epidemiological link support the hypothesis of nosocomial transmission. No additional cases of N-gene dropout were detected amongst inpatients or healthcare workers (HCWs) despite intensive surveillance including weekly, rostered, routine testing.[7] This result is likely due to enhanced infection prevention measures in-place during the study period, including universal N95 usage by HCWs and high vaccination-uptake rates (≥80%) among inpatients and HCWs.[9]

The potential of nosocomial SARS-CoV-2 transmission from cases of N-gene dropout is not purely theoretical. Clinicians and diagnostic laboratories need to be vigilant for such cases because emergence of novel SARS-CoV-2 variants with the potential to escape existing diagnostic tests remains inevitable. Test interpretation is critical. According to the manufacturer instructions for the Cepheid GeneXpert-Xpert-Xpress test kit, cases with discrepant results (negative on the N2-gene gene target but positive on the E-gene gene-target) should have been reported as presumptive positive with a recommendation for repeated testing.[10] Had this interpretation been used, the patient would have been isolated upon admission and the additional period of transmission risk would not have occurred, despite the N-gene dropout. SARS-CoV-2 diagnostic assays should ideally be based on ≥2 gene targets, and healthcare institutions need to maintain a robust molecular surveillance system to detect emergent diagnostic escapes. Reliable diagnostic detection of SARS-CoV-2 is crucial for containment of COVID-19 and prevention of spread within healthcare facilities.

Acknowledgments.

Financial support. This work was not grant funded. Funding for consumables was provided by the Singapore General Hospital. K.K.K.K. is supported by the Singapore National Medical Research Council Research Training Fellowship.

Conflicts of interest. The authors report no conflicts of interest relevant to this report.

References

1. Prabhu RM, Firestone MJ, Bergman K, *et al.* Use of serial testing to interrupt a SARS-CoV-2 outbreak on a hospital medical floor—Minnesota, October–December 2020. *Infect Control Hosp Epidemiol* 2022. doi: 10.1017/ice.2022.40.
2. Chen Y, Han Y, Yang J, Ma Y, Li J, Zhang R. Impact of SARS-CoV-2 variants on the analytical sensitivity of rRT-PCR assays. *J Clin Microbiol* 2022; 60:e0237421.
3. Hasan MR, Sundararaju S, Manickam C, *et al.* A novel point mutation in the N gene of SARS-CoV-2 may affect the detection of the virus by reverse transcription-quantitative PCR. *J Clin Microbiol* 2021;59: e03278–20.
4. Ziegler K, Steininger P, Ziegler R, Steinmann J, Korn K, Ensser A. SARS-CoV-2 samples may escape detection because of a single point mutation in the N gene. *Euro Surveill* 2020;25:2001650.
5. Ko KKK, Abdul Rahman NB, *et al.* SARS-CoV-2 N gene G29195T point mutation may affect diagnostic reverse transcription-PCR detection. *Microbiol Spectr* 2022;10:e0222321.
6. Wee LE, Conceicao EP, Sim JX, *et al.* Utilization of rapid antigen assays for detection of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) in a low-incidence setting in emergency department triage: does risk-stratification still matter? Infect Control Hosp Epidemiol 2021. doi: 10.1017/ice.2021.407.
7. Wee LEI, Conceicao EP, Aung MK, *et al.* Rostered routine testing for healthcare workers and universal inpatient screening: the role of expanded hospital surveillance during an outbreak of coronavirus disease 2019 (COVID-19) in the surrounding community. Infect Control Hosp Epidemiol 2021. doi: 10.1017/ice.2021.366.

8. Wee LE, Ko KK, Conceicao EP, *et al.* Linking sporadic hospital clusters during a community surge of the severe acute respiratory coronavirus virus 2 (SARS-CoV-2) B.1.617.2 delta variant: the utility of whole-genome sequencing. Infect Control Hosp Epidemiol 2022. doi: 10.1017/ice.2022.106.
9. Liang En W, Ko KK, Conceicao EP, *et al.* Enhanced infection-prevention measures including universal N95 usage and daily testing: the impact on SARS-CoV-2 transmission in cohorted hospital cubicles through successive delta and omicron waves. *Clin Infect Dis* 2022:ciac320.
10. Xpert Xpress SARS-CoV-2 test: package insert. Cepheid website. https://www.cepheid.com/Package%20Insert%20Files/Xpert%20Xpress%20SARS-CoV-2%20Assay%20ENGLISH%20Package%20Insert%20302-3787%20Rev.%20B.pdf. Published 2021. Accessed June 6, 2022.

Lack of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) transmission from a healthcare worker to a cohort of immunosuppressed patients during the SARS-CoV-2 omicron variant surge, California, 2022

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To the Editor—During the coronavirus disease 2019 (COVID-19) surge in January 2022 caused by the severe acute respiratory coronavirus virus 2 (SARS-CoV-2) o (omicron) variant, more infections among healthcare workers (HCWs) were documented than at any other point in the COVID-19 pandemic. Transmission from infected HCWs to other HCWs and patients is a noteworthy concern. The risk of an HCW acquiring COVID-19 from another HCW is reportedly 3 times higher than contracting it from patients.[1] Given that not all infections are detected, it is critically important that during surges, all infection controls (administrative, engineering, and personal protective equipment [PPE]) are optimized to protect patients and HCW colleagues.[2] We report a case in which a highly infectious HCW working with profoundly immunosuppressed patients did not transmit SARS-CoV-2 to patients despite multiple close interactions. Our data analysis was approved under the Stanford University Institutional Review Board (IRB) through expedited review.

The index HCW was vaccinated with BNT162b2 in December 2020 and January 2021 and received a booster vaccination in October 2021. The HCW was in their usual state of health and had a negative routine screening rtPCR anterior nares swab (self-collected; Color Health, Inc) on the Monday of the week of infection; medical school personnel were required to test weekly regardless of symptoms or exposure history. The following day, the HCW felt mild fatigue in the morning, which resolved soon after awakening. The HCW proceeded to work and performed physical exams on 7 patients over the course of the day. The patients were moderately to severely immunocompromised. All but 1 patient had undergone solid-organ transplantation, and 1 patient had undergone heart transplantation on day 1 following the interaction with the infectious HCW. The HCW began to feel symptoms while on the wards, including severe fatigue, diffuse sweating, and chills, which prompted the HCW to leave the wards and proceed with rapid rtPCR testing via a nasopharyngeal sample. The HCW went home to isolate. The rtPCR returned positive within 2 hours (cycle threshold value [Ct], 15.9). The HCW had also examined 7 patients the day before symptom onset. Of these patients, 3 were examined both days. The HCW worked closely with another HCW for ~8 hours the day before onset of symptoms and for ~6 hours on the day of symptom onset.

The index HCW reported wearing an N95 respirator during all patient encounters and during all other activities during the workday. Both HCWs wore fit-tested N95 respirators in the presence of each other. Patients were not masked during encounters, except one who was wearing both an N95 respirator and a surgical mask. Patient encounters were reported as being <2 m distant but <15 minutes in duration. None of the patients were in airborne isolation because they had not reported any recent COVID-19 symptoms or known exposures. Although the index HCW's sample was not subjected to whole-genome sequencing, most samples were SARS-CoV-2 omicron variant during this period. We tested all patients except for 2 who had been discharged. All available SARS-CoV-2 tests returned negative (Table 1). The second HCW who was exposed also tested negative on multiple follow-up tests.

This report is important for several reasons. First, the HCW had a negative weekly screening test on Monday with only mild fatigue that resolved upon awaking on the next day. This type of symptomatology would not be accurately picked up on a screening symptom survey given high frequency of fatigue among HCWs at baseline.[3] Second, the patients themselves were not adequately masked, which emphasizes the need for HCWs themselves to wear high-quality masks as PPE and source control, such as N95 respirators. Third, these patients were immunocompromised, putting them at higher risk of severe infection and death (as high as 13%–20%) if they were to contract SARS-CoV-2.[4,5]

We hypothesize many possible reasons why the index case did not transmit to patients or other HCWs. First, the index case was wearing an N95 respirator, which may have provided superior source control compared to surgical masks.[6] Both HCWs who were in close contact for 2 days were wearing N95 respirators during all encounters. Some have argued that the universal use of N95 respxirators during COVID-19 surges is an important measure to reduce in-hospital transmission.[2]

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Cite this article: Karan A, Ferguson J, and Salinas JL. (2023). Lack of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) transmission from a healthcare worker to a cohort of immunosuppressed patients during the SARS-CoV-2 omicron variant surge, California, 2022. *Infection Control & Hospital Epidemiology*, 44: 156–157, https://doi.org/10.1017/ice.2022.175

https://doi.org/10.1017/ice.2022.170 Published online by Cambridge University Press