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Letter to the Editor

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 repeated 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 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

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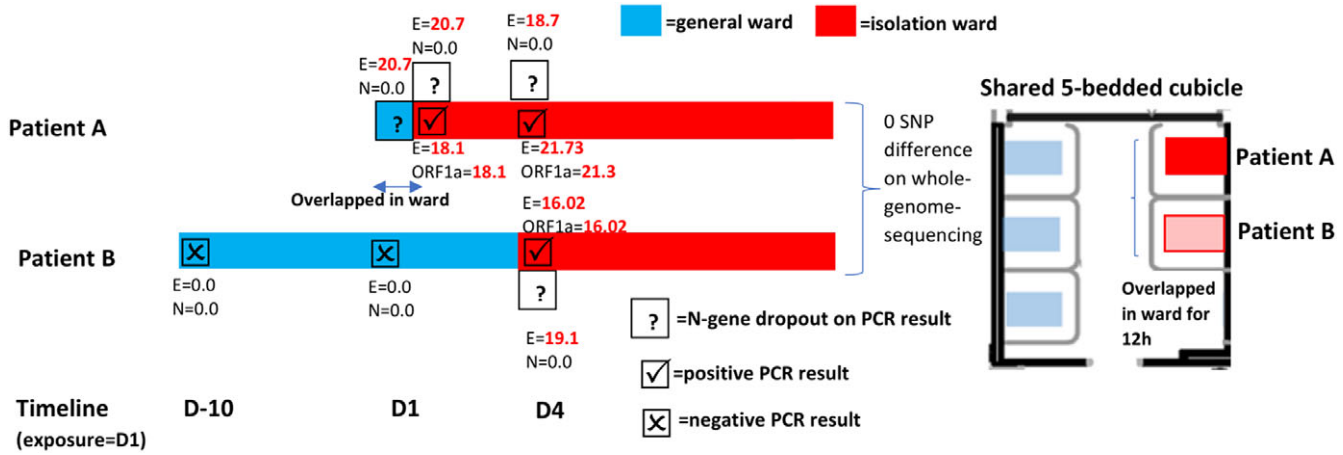

Fig. 1. Nosocomial transmission arising from a case of N-gene diagnostic escape.

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.

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