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Brief Report

Digital Droplet PCR to Track SARS-CoV-2 Outbreak in a Hospital Transitional Care Unit

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Numerous outbreaks have been reported in healthcare settings during the SARS-CoV-2 pandemic. Transmission occurs mainly via respiratory droplets and possibly contaminated surfaces or aerosols. Rapid propagation of SARS-CoV-2 within healthcare settings has raised the possibility of “super-spreader” events.

This report describes an outbreak that occurred within a single unit at a large urban hospital in Calgary, Alberta, Canada during Fall of 2020 in a transitional unit for patients awaiting long-term care.

METHODS

Outbreak identification and management

The initial case was a regular visitor who tested positive on November 17, 2020. The patient associated was isolated and screened. The following day they tested positive, and a multi-disciplinary outbreak management team was established. As all patients had been on the unit for weeks to months, any new cases were considered nosocomial. Detailed epidemiologic investigation was pursued and key outbreak control measures were implemented including prevalence testing of all patients and healthcare workers (HCW) every 3-5 days, precautions for symptomatic positive patients and their contacts, unit closure to admission or transfer, enhanced cleaning, discontinuation of group activities, and strict visitor restrictions. Any positive patients were transferred to designated COVID units.

Laboratory tests and environmental sampling

Patient and HCW testing were done using reverse-transcription PCR (RT-PCR) as per Alberta Precision Laboratories’ (APL) standard protocols. Genomic sequencing was performed on select samples to determine lineage by the Alberta Public Health Laboratory external to this study. A total of 97 environmental samples were taken throughout the unit by using flocculated swabs to sample each area of interest the same number of times, placing into Dulbecco’s Modified Eagle Medium (DMEM), and freezing at -80 °C before being extracted for analysis.

In addition to routine diagnostic testing, patient and environmental samples were tested using the Bio-Rad SARS-CoV-2 ddPCR Kit (Bio-Rad) with an AutoDG and QX200 ddPCR system (Bio-Rad). Data was analyzed in QX Manager 1.2 Standard Edition. Results were reported as copies/μL of N1 and N2 genes in the extracted RNA.

RESULTS

From November 19 to December 29, 2020, there were 34 cases (21 patients, 12 HCWs, 1 visitor). Despite immediate implementation of
outbreak measures, by the sixth day, 14 patients and 3 HCWs were positive (Fig 1). Due to the rapid spread and high attack rate, the unit was closed on November 27; all patients were transferred to COVID units, and all HCWs were furloughed for 14 days. Seventy percent of patients tested positive (mean age = 84 years, 45% male). Two patients passed away, while 42% required oxygen therapy. There was progressive geographic spread starting from the index case room suggesting rapid person-to-person transmission (Fig 2A).

There was an incomplete uptake for HCW prevalence screening, and 12 of 49 HCWs tested positive during the outbreak; 10 cases were determined to be hospital-acquired. None required hospitalization, and two remained asymptomatic.

Genomic sequencing performed on select patient samples shared a common B.1.438 phylogeny (data not shown). As multiple lineages were present at the time, these data suggest one or few sources of introduction.

From the 97 environmental samples, 7% were positive for SARS-CoV-2 genetic material (n = 7) (Fig 2A). Four were from common areas and/or items (walkers and chairs). The swab of a blanket warmer, sink, and door handle inside the clean utility room was also positive.

Several of the early patient case samples were quantified using ddPCR (Fig 2B). Viral gene load varied, with some having very high levels of virus.

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**Fig 1.** Outbreak timeline. P = patient, S = staff. Boxes indicate illness duration; yellow = patient, purple = health care worker, green = visitor. Faded boxes indicate asymptomatic illness. +/− indicate results of the RT-PCR test for COVID-19 taken on that date. Dark circles indicate patients who required oxygen on that date, and red boxes indicate the date patients became deceased. Red stars indicate patients who tested to have a very high viral load as per ddPCR testing (Figure 2). Blue line indicates the date of unit closure (Color version of the figure is available online.).

**Fig 2.** (A) Results of environmental swab testing by ddPCR. Blue indicates negative for SARS-CoV-2 genetic material. Yellow indicates low detection (either N1 or N2 detected at 0.2-0.5 cp/μL). Orange indicates medium detection (either N1 or N2 detected at >0.5 cp/μL). Red indicates high detection (both N1 and N2 detected, >0.2 cp/μL). (B) Outbreak tracking by viral gene load in patient swabs as measured by ddPCR. Viral gene load (red circles being the highest, gray circles being the lowest number of gene copies detected) is plotted by patient room number by the date when the sample was taken. Centre of circles indicates patient location and date of sampling. Blue line indicates the date of unit closure. On the room map, red stars indicate rooms with positive patients during the outbreak, while red filled circles indicate the location of high viral load presumptive “super-spreader” patients, and red open circles indicate the location of environmental samples positive for SARS-CoV-2, close to these patients (Color version of the figure is available online.).
DISCUSSION

Cases increased rapidly with an epidemic curve suggesting a common source of exposure. ddPCR testing demonstrated several early cases had high viral burden corroborating rapid spread from few sources. The patient population coupled with unit layout were significant contributing factors. Many patients had underlying dementia and prone to wandering. Similar challenges have been observed in long term care and combined nursing facilities.6

During the outbreak, surface sanitation was performed multiple times daily. Staff were also required to wear PPE, and were asked to wash and sanitize their hands frequently in addition to following social distancing. Most environmental samples were negative for SARS-CoV-2, including a room recently occupied by a COVID-19 patient, indicating cleaning practices were sufficient for decontamination. A few potential sources of spread included a clean utility room as well as shared common use items which had viral genetic material identified by ddPCR. These results indicate that fomite transmission may have played a role in this outbreak. Other studies have reported the importance of contaminated surfaces in SARS-CoV-2 transmission.7

This study was novel in its use of ddPCR. RT-PCR testing of patient samples has been the primary method used to identify COVID-19 cases.7 ddPCR differs in that the reaction is separated into droplets containing template and the components for the RT-PCR reaction. Each droplet is then measured for absolute fluorescence at the end-point, and using Poisson distribution, allows for end-point quantification without standard curves in a way that is less sensitive to reaction efficiency and inhibitors. Because of this, ddPCR may provide diagnostic advantages in sensitivity, quantitative capability, and robustness to inhibition for samples of lower quality or those with lower viral titers such as environmental swabs.8 In this study, ddPCR was able to identify patients with high viral loads and previously unknown points of environmental contamination. While Ct values from RT-PCR tests can give some indication of viral loads, they are not as exact in their estimations as ddPCR without the use of a standard curve, which are typically not performed in routine diagnostics. Identification of “super-spreader” individuals and areas of potential environmental risk during an outbreak could allow for better tracking and outbreak management.

Several key learning points were shared with frontline staff and management, including careful patient and HCW symptom screening, early precaution implementation and testing, increased vigilance around cleaning, and removal of common items. Patient population characteristics, unit infrastructure, and novel diagnostic technology such as ddPCR may be useful in understanding outbreak sources.

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