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Digital droplet PCR to track SARS-CoV-2 outbreak in a hospital transitional care unit

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Running Title: Digital droplet PCR SARS-CoV-2 outbreak

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Abstract

We describe a large outbreak of SARS-CoV-2 on a transition unit composed of elderly patients awaiting placement. Environmental and patient sample analyses using ddPCR suggested possible fomite transmission and high viral burden source from a few individual patients. This outbreak illustrates challenges inherent to this specific patient population.

Keywords: SARS-CoV-2, COVID-19, outbreak, long term care, ddPCR

Introduction

Numerous outbreaks in healthcare settings during the SARS-CoV-2 pandemic have been reported. Transmission occurs mainly via respiratory droplets and possibly contaminated surfaces or aerosols.[1] Rapid propagation of SARS-CoV-2 within healthcare settings has raised the possibility of "super-spreader events".[2,3]

This report describes an outbreak that occurred within a single unit at a large urban hospital in Calgary, Alberta, Canada during fall 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 17th, 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 to 5 days, precautions for symptomatic positive patients and their contacts, unit closure to admission or transfers, 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 was done using reverse-transcription PCR (RT-PCR) as per Alberta Precision Laboratories' (APL) standard protocols.[4] Genomic sequencing was performed on select samples to determine lineage at Alberta Health Bioinformatics. A total of 97 environmental samples were taken throughout the unit by using floc swabs to thoroughly swab 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.[5]

In addition to testing through APL, patient and environmental samples were tested using the Bio-Rad SARS-CoV-2 ddPCR Kit (Bio-Rad, Pleasanton, CA, USA) with an AutoDG and QX200 ddPCR system (Bio-Rad, Pleasanton, CA). 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 (Figure 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 became 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 spread (Figure 2A).

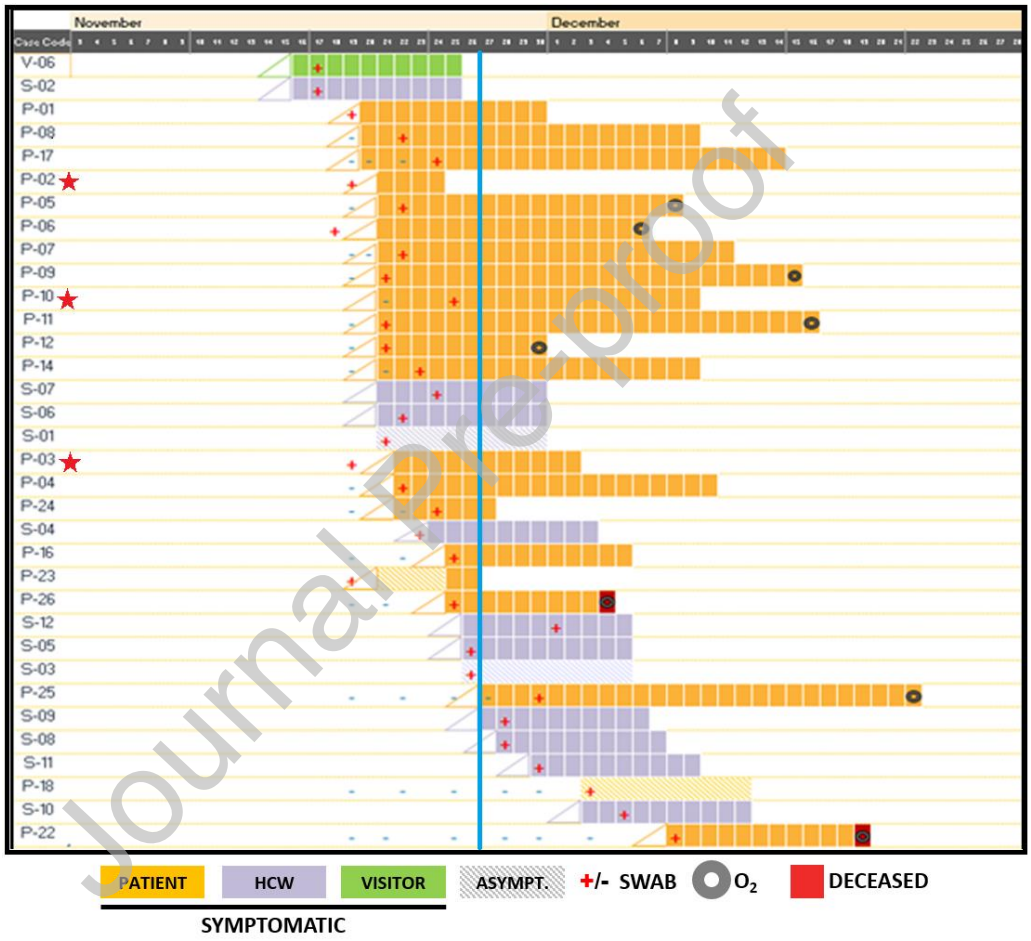

Figure 1: Outbreak timeline. P=patient, S=staff. Boxes indicate illness duration; yellow = patient, purple = health care worker, green = visitor. Faded boxes indicate asymptomatic illness. -/+ indicate results of RT-PCR test for COVID-19 taken on that date. Dark circles indicate patients who required oxygen on that date, and red boxes indicate 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 date of unit closure.

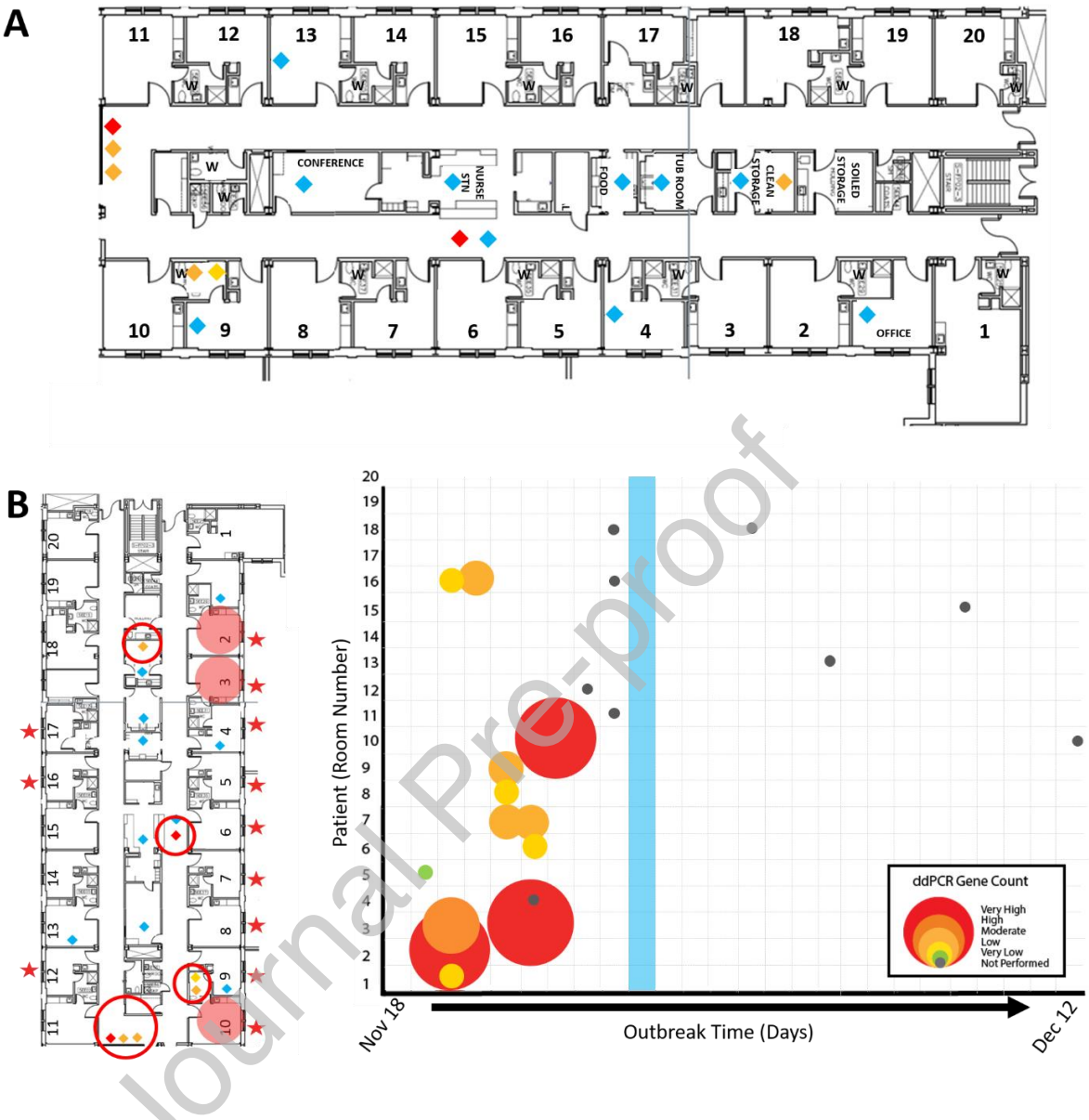

Figure 2: A) Results of environmental swab testing by ddPCR. Blue indicates negative for SARS-CoV-2 genetic material. Yellow indicates low detection (either N1 or N2 detected at 0.2-0.5 cp/µL). Orange indicates medium detection (either N1 or N2 detected at >0.5 cp/µL). Red indicates high detection (both 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 highest, grey circles being lowest number of gene copies detected) is plotted by patient room number by date when sample was taken. Centre of circles indicate patient location and date of sampling. Blue line indicates 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 "super-spreader" patients, and red open circles indicate the location of environmental samples positive for SARS-CoV-2, close to these patients.

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

Genomic screening showed samples shared a common phylogeny (B.1.438). As multiple lineages were present at the time, this supports one or few sources of introduction.

From the 97 environmental samples, 7% were positive for SARS-CoV-2 genetic material (n=7) (Figure 2A). Four were from common areas/items (walkers and chairs). The swab of the 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 (Figure 2B). Viral gene load varied, with some having very high levels of virus.

Discussion

Cases rapidly increased with an epidemic curve pattern suggesting a common source of exposure. ddPCR testing showed several early cases had high viral burden, corroborating rapid spread from few sources. The specific patient population coupled with unit layout were significant contributing factors. Many patients had underling dementia and wandered; these patients were also unable to comply well with masking, hygiene, and physical distancing. 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 came back negative for SARS-CoV-2 genes, including a room recently occupied by a COVID-19 patient, indicating cleaning practices were mostly sufficient for decontamination. However, a few potential sources of spread including a clean utility room accessed only by staff as well as shared common use items had viral genetic material, potentially indicating improper hygiene practices. The 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.[1]

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 endpoint, 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 titres such as environmental swabs.[8] In this study, ddPCR was able to identify patients with high viral loads along with 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 was typically not run during pandemic 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 and unit infrastructure should be taken into account for control, and novel diagnostic technology may be useful in understanding outbreak anatomy.

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