Evidence from Whole Genome Sequencing of Aerosol Transmission of SARS-CoV-2 almost Five Hours after Hospital Room Turnover

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Short Report

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Abstract

Experimental evidence suggests that SARS-CoV-2 remains viable within aerosols with a half-life of approximately 1-3 hours, though changes in aerosol microenvironment may shorten viability to minutes. However, it remains unclear how long airborne SARS-CoV-2 can transmit infection. Whole genome sequencing of nasopharyngeal samples obtained from patients on an outbreak unit suggested in-room transmission of the delta variant, AY3 lineage, of SARS-CoV-2 to two patients admitted 1 hour, 43 minutes and 4 hours, 45 minutes after discharge of an asymptomatic infected patient. These findings suggest that airborne SARS-CoV-2 may transmit infection for nearly 5 hours, even in a hospital setting.

Introduction

Airborne transmission is a major source of SARS-CoV-2 infection. SARS-CoV-2 has been isolated from air samples in rooms of infected patients and in hospital locations remote from the care of patients with COVID-19, such as nurses stations on non-COVID units. In one hospital outbreak, whole genome sequencing (WGS) from air samples, staff, and patients implicated aerosol transmission as the likely mechanism of transmission. Experimental evidence suggests that SARS-CoV-2 remains viable within aerosols with a half-life of approximately 1–3 hours, though changes in aerosol microenvironment may shorten viability to minutes. However, it remains unclear how long airborne SARS-CoV-2 can transmit infection. Here we present evidence from WGS suggesting in-room transmission of SARS-CoV-2 to two patients admitted 1 hour, 43 minutes and 4 hours, 45 minutes after discharge of an asymptomatic infected patient.

Methods

This is a molecular epidemiological analysis of a previously reported case series from a single inpatient unit at VA Boston Healthcare System (VABHS) with nosocomial transmission of the SARS-CoV-2 delta variant. The outbreak occurred in July of 2021. Viral WGS was performed on nasopharyngeal swab samples, as described, and used to characterize the chain of transmission between individuals. The IRB at VABHS deemed this work a quality improvement study and waived IRB approval.

Results

WGS identified the SARS-CoV-2 delta variant, AY3 lineage, and subdivided all eight individuals from the outbreak into two clusters of four (Figure). Samples from each cluster demonstrated sequence identity, and Cluster 2 differed from Cluster 1 by a single nucleotide polymorphism (SNP), the reversion mutation T29742G. In contrast, WGS on contemporary samples from two VABHS inpatients who were not on the outbreak unit and two from VA Connecticut differed from those in the outbreak clusters by 2 to 6 SNPs (Supplementary Table 1).

Cluster 1
Patient A, the presumed index case, was hospitalized for 24 days on the outbreak unit, overlapping the remaining seven persons in the outbreak clusters. Patient A was diagnosed with asymptomatic COVID-19 during a mandatory pre-discharge PCR test (Ct = 26; Outbreak Day 0). He had walked unmasked throughout the unit and eventually required a designated nursing assistant (Person B). Through contact tracing, Person B was found to be negative by antigen testing on Outbreak Day 2 but was diagnosed with asymptomatic COVID-19 on Outbreak Day 5 (Ct = 18). Patients C and D had symptomatic hospital-acquired COVID-19 on Outbreak Days 4 and 10, respectively. Both were admitted to the unit prior to the outbreak, had a negative PCR within 24 hours of admission, and converted on the unit.

**Cluster 2**

All four persons in Cluster 2 spent time in the same hospital room. Patient E was hospitalized for 15 days concurrent with Patient A’s hospitalization, though in a different room, and was discharged three days before Patient A’s COVID-19 diagnosis. Contact tracing identified asymptomatic COVID-19 (Ct = 25) in Patient E eight days after discharge.

Patient E was discharged at 11:00 AM on Outbreak Day − 3. Two patients without prior contact with Patient E were admitted that same day to the room previously occupied by Patient E: Patient F at 12:43 PM and Patient G at 3:45 PM. Both patients had negative COVID-19 tests four days prior to admission. Patient F was discharged one day following admission (Outbreak Day − 2) and diagnosed with asymptomatic COVID-19 (Ct = 29; Outbreak Day 1) four days after admission. Patient G was diagnosed with presymptomatic COVID-19 (Ct = 23) three days after admission, became symptomatic one day later, and transmitted SARS-CoV-2 to a visiting family member, who was diagnosed six days later with presymptomatic COVID-19 (Ct = 15; Outbreak Day 6).

Contact tracing was negative in 168 masked staff member, 6 visitors, and 38 additional patients. Air turnover in this room was measured at 6 per hour prior to the outbreak.

**Discussion**

WGS may be helpful in identifying transmission chains during hospital outbreaks. One cluster of transmissions on this outbreak unit was from Patient A to the rest of Cluster 1 - his nursing assistant (Person B) and Patients C and D. All four persons were infected with identical versions of the SARS-CoV-2 Delta variant (AY3 lineage). Because Patient A and Patient E were hospitalized concurrently, it appears likely that Patient A also infected Patient E, and a reversion mutation (T29742G) occurred before or during this transmission event. Transmission from other sources was improbable based on extensive contact tracing and the nearly identical viral sequences identified in the two clusters. It is unlikely that Patient A independently infected Patients E, F and G because this would have required three identical reversion mutation events, a statistical improbability. More likely, SARS-CoV-2 in lingering aerosols or from aerosol-contaminated surfaces from Patient E remained viable for hours before infecting Patients F and G. This occurrence would imply that airborne SARS-CoV-2 is not only viable for hours in aerosols but is also capable of transmission.
Limitations of this study are its observational nature and the lack of sufficiently frequent SARS-CoV-2 testing to identify the onset of infection more precisely. Likewise, the measurement of air turnover was not contemporaneous with the outbreak; therefore, we cannot be certain about the level of room ventilation during the outbreak.

**Declarations**

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**References**

Figure 1

Proposed Transmission Chain Based on Whole Genome Sequencing (WGS) of SARS-CoV-2. WGS of nasopharyngeal samples from eight persons identified the AY3 lineage of the Delta Variant. WGS was identical for the four individuals depicted in green circles (Cluster 1) and included the G29742T mutation.

- A → B → C → D
- E → F → G → H

Cluster 1: G29742T
Cluster 2: T29742G

Discharged 11:00 AM
Admitted 12:43 PM
Admitted 3:45 PM
WGS for the four individuals depicted in blue circles (Cluster 2) differed from that of Cluster 1 by one single nucleotide polymorphism (SNP): T29742G. Colored rectangles surrounding the circles reflect single or shared hospital rooms. Black arrows depict transmission events. The red arrow depicts a reversion mutation (T29742G) to the original Wuhan-Hu-1 strain before or during transmission from Patient A to Patient E. Patient E was diagnosed with asymptomatic infection eight days following discharge. Patients F and G were admitted to the same hospital room 1 hour and 43 minutes and 4 hours and 45 minutes, respectively, after Patient E was discharged. Lingering aerosol (red dots) from Patient E may have infected both Patient F and Patient G, who later infected a visiting family member, Person H. WGS on Person H demonstrated one new SNP - A24410G - which was also identified for the first time in a sample from Patient G six days after their initial sample.

**Supplementary Files**

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- [SupplementaryTable.xlsx](#)