Detection of Environmental Spread of SARS-CoV-2 and Associated Patient Characteristics

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Abstract: Understanding transmission of SARS-CoV-2 informs infection prevention practices. Air sampling devices were placed in patient hospital rooms for consecutive collections with and without masks. With patient mask use, no virus was detected in the room. High viral load and fewer days from symptom onset were associated with viral particulate dispersion.

Key Words: COVID-19, Viral Pneumonia, Infection Prevention and Hospital Epidemiology
Background: Public health and infection control strategies regarding the COVID-19 pandemic are informed by our understanding of the spread of the SARS-CoV-2 virus. Viral spread, as defined by dispersion of viral particulates, correlates to the period of infectiousness for person-to-person transmission.\(^1\) Viral load rapidly declines during the first week after symptom onset, and while shedding of viral particles may persist for several weeks, live virus has been cultured only early in the course of infection.\(^1,2\) The significance of transmission via aerosolized droplet nuclei (<5 μm in diameter), which may remain airborne as infectious particles for several hours has prompted investigation.\(^3-5\) However, a meta-analysis described large respiratory droplets as the primary mode of transmission, in accordance with CDC guidelines.\(^2,6\) Furthermore, certain patient characteristics and biomarkers have been associated with worse disease course and higher viral loads.\(^6,7\) With limited exceptions, the current literature infrequently describes patient clinical features associated with environmental detection of virus.\(^4\) We measured the impact of source-control mask use and the associated patient clinical characteristics on environmental burden of SARS-CoV-2 in hospitalized patients.

Methods: This was a single-arm non-randomized control trial to study the effect of mask use on environmental spread of SARS-CoV-2 virus. Participants included SARS-CoV-2 positive adults admitted to a single academic center from April 13 - May 22, 2020. Participants provided written consent as approved by Wake Forest institutional IRB 00064866. Patients were selected on a rolling basis using the Epic electronic health record (EHR) to identify adults with clinical laboratory confirmed reverse transcription-polymerase chain reaction (RT-PCR) positive status for SARS-CoV-2 (Epic Systems Co, Wisconsin, USA). Patients requiring mechanical ventilatory support at time of enrollment were excluded.

Sample collection took place in hospital rooms with heating, ventilation and air conditioning (HVAC) systems exchanging room air 6 times per hour using MERV-14 filters with capacity to filter 90% of particles 1-3 microns in diameter.\(^8\) Following enrollment a nasopharynx (NP) swab was obtained from each participant for viral load prior to air sampling. Andersen air
sampling devices and surface sedimentation plates were placed at three locations in patient hospital rooms (Figure 1). To study the effect of source-control mask use on environmental contamination of virus, two 30-minute air sample runs were obtained for each study participant. During the first run, the patient was not wearing a mask, followed by a second run with patients wearing a non-medical 3-ply procedural mask (Haowei Weiye Technology Development, Harbin, China). Viral transport media (VTM) on sedimentation plates from Anderson air samplers were pooled from stages 1 and 2 (filter sizes ≥5 μm) and stages 3 - 6 (filter sizes <5 μm) to separate large droplets from aerosols. Following a standard pattern, a 3 by 3 inch cutting from the center of patient masks was stored in VTM. Participants had 20 samples each: 9 samples from both environmental sampling runs (3 stations with 1 surface sample and 2 pooled samples from air sampling devices), patient mask and NP swab. SARS-CoV-2 RNA was extracted from VTM, reverse transcribed into cDNA for viral amplification, and quantified by qPCR with CDC N1 and N2 detection assays. The viral load was reported as the threshold cycle (Ct), with lower Ct values representing higher viral loads. Our lower detection limit was approximately 75 viral genome copies per site measured, which falls below the infectious dose of coronaviruses.9 Clinical characteristics including, comorbidities and biomarkers were recorded from the EHR and patient interview. Using R statistical software, we assessed the association of NP viral load and days since symptom onset with positive detection in the mask or environment using 2-sample t-tests. Random Forest imputation was used to impute two missing symptom onset values. We used Firth logistic regression in independent models to investigate the odds of positive detection of viral spread with clinical factors of interest (e.g. diabetes, hypertension, etc.), each controlling for days since symptom onset. This model accurately detects significance but is imprecise with magnitude of association in small samples.

Results: The study population included 26 adults mean age 58.4 (SD, 15.9), male 62%, with comorbidities including hypertension 73%, obesity 62%, type 2 diabetes 46%, and heart disease 35%. Viral spread, as defined by detection of viral particulate dispersion in the
patient mask or environment, was present in 11 of 26 patients (42%) (Table 1). Viral particles in large respiratory droplets were recovered adjacent to the head from 2 of 26 patients (8%; droplet sizes ≥5 um) who were closer to symptom onset (2 and 4 days). No aerosol sized particles were detected in air samplers for masked or unmasked runs. No virus was recovered from air samplers or surface plates from runs with patients wearing masks. Detection of virus in masks or environment was significantly associated with higher NP viral loads (mean (95% CI) = 20.6 (17.9, 13.2) for positive emission and 31.3 (29.1, 33.6) for negative emission; \( p < 0.001 \)). Furthermore, viral dispersion was linked to fewer days from symptom onset (mean (95% CI) = 5.09 (2.03, 8.15) for positive emission patients versus 10.23 (7.61, 12.85) for negative emission patients; \( p = 0.015 \)). From our Firth logistic regression models controlling for time from symptom onset, clinical features associated with viral spread, as defined above, included diabetes (\( p = 0.02 \)), hypertension (\( p = 0.04 \)), heart disease (\( p = 0.01 \)), obesity (\( p = 0.06 \)), acute kidney injury (AKI) (\( p = 0.01 \)), and leukocytosis (\( p = 0.02 \)). There was no significant difference between viral spreaders and non-spreaders in vital signs or level of care during collections (Supplement).

Discussion:

In a limited sample of \( n = 26 \) hospitalized patients, SARS-CoV-2 was detected only within large respiratory droplet range in unmasked patients (≤2 ft from head of patient), using air sampling methods validated on airborne viruses. These findings support the use of contact and droplet precautions in routine clinical scenarios in hospitals. We demonstrated patient mask use stopped environmental dispersion of virus over 30-minute collections, which confirms low risk of viral transmission from infected hosts while using masks. Viral load (\( \text{Ct} \leq 25 \)) followed by shorter duration from symptom onset (≤8 days), had the strongest association with spread of viral particles, which supports recent CDC strategies for decreased quarantine time following infection. Clinical factors associated with severe disease course were linked to viral spread including, diabetes,
hypertension, heart disease, obesity, AKI, and leukocytosis. Given limitations on sample size we are unable to conclude whether comorbidities are independently associated with increased transmission, or if people with more comorbidities have increased viral shedding due to greater severity of infection. The other main limitation of this study was decreased sensitivity of detection limits compared to other environmental sampling studies. Our findings may lead to future investigations including use of patient masks as a cost-effective method to describe patient characteristics associated with viral spread.
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Table 1: Clinical Features Associated with Spread of SARS-CoV-2

| DEMOGRAPHICS                  | Overall (n=26) | Positive Spread (n=11) | Negative Spread (n=15) | p-value* |
|------------------------------|---------------|------------------------|------------------------|----------|
| Age (mean (SD))              | 58.35 (15.90) | 63.18 (10.28)          | 54.80 (18.54)          | 0.21     |
| Sex - Male (%)               | 16 (61.5)     | 8 (72.7)               | 8 (53.3)               | 0.35     |
| Race/ethnicity (%)           |               |                        |                        | 0.625    |
| Black                        | 5 (19.2)      | 3 (27.3)               | 2 (13.3)               |          |
| Hispanic                     | 12 (46.2)     | 5 (45.5)               | 7 (46.7)               |          |
| White                        | 9 (34.6)      | 3 (27.3)               | 6 (40.0)               |          |
| CLINICAL FEATURES            |               |                        |                        |          |
| NP Viral Load Ct (mean (SD) [95%CI]) | 26.76 (6.83) | 20.55 [17.9, 13.2]    | 31.32 [29.1, 33.6]    | <0.001   |
| Days from Symptom Onset (mean (SD) [95%CI]) | 8.06 (5.47)  | 5.09 [2.03, 8.15]     | 10.23 [7.61, 12.85]   | 0.015    |
| Days from Lab Diagnosis (mean (SD)) | 3.58 (3.50)  | 1.91 (1.58)            | 4.80 (4.04)           | 0.034    |
| COMORBIDITIES                |               |                        |                        |          |
| Type 2 Diabetes (%)          | 12 (46.2)     | 9 (81.8)               | 3 (20.0)               | 0.024    |
| Hypertension (%)             | 19 (73.1)     | 11 (100.0)             | 8 (53.3)               | 0.039    |
| Heart Disease (%)            | 9 (34.6)      | 7 (63.6)               | 2 (13.3)               | 0.008    |
| Obesity (%)                  | 16 (61.5)     | 8 (72.7)               | 8 (53.3)               | 0.058    |

LABORATORY
| BIOMARKERS               |       |      |       |      |
|-------------------------|-------|------|-------|------|
| Acute Kidney Injury (%) | 13 (50.0) | 9 (81.8) | 4 (26.7) | 0.012 |
| Leukocytosis (%)        | 10 (38.5) | 6 (54.5) | 4 (26.7) | 0.016 |

†Spread defined by detection of SARS-CoV-2 viral particles in the patient mask or hospital room.

*p-values for demographics, viral load Ct, days from symptom onset, and days from lab diagnosis are from univariate t-tests. All other p-values are from logistic regression model using Firth’s bias correction, controlling for days from symptom onset.
Figure 1 Legend

Figure 1A: Unmasked patient sample collection with PCR detection of virus in Nasopharynx Swab (A) and samplers located at 2 feet, 5-6 feet, and 8-10 feet (B, C, and D) from the patient’s head.

Figure 1B: Masked patient sample collection with PCR virus detection in Patient Mask (a) and sampler placement same as above (b, c, and d). Sampler stations included air sampler and surface sedimentation plate.
**Figure 1A**

**Unmasked**

- \( A \) Nasopharynx Swab
  - \( n=23/26 \) (88.5%) detected

- \( B \) 2 Feet Sampler
  - \( n=2/26 \) (7.7%) detected

- \( C \) 5-6 Feet Sampler
  - \( n=0/26 \) (0%) detected

- \( D \) 8-10 Feet Sampler
  - 0% detected

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**Figure 1B**

**Masked**

- \( a \) Patient Mask
  - \( n=10/26 \) (38.5%) detected

- \( b \) 2 Feet Sampler
  - \( n=0/26 \) (0%) detected

- \( c \) 5-6 Feet Sampler
  - 0% detected

- \( d \) 8-10 Feet Sampler
  - 0% detected