Community-Based SARS-CoV-2 Testing Using Saliva or Nasopharyngeal Swabs to Compare the Performance of Weekly COVID-19 Screening to Wastewater SARS-CoV-2 Signals

Zhengchun Lu, Amanda E. Brunton, Maedeh Mohebnasab, Anthony Deloney, Kenneth J. Williamson, Blythe A. Layton, Scott Mansell, Alice Brawley-Chesworth, Peter Abrams, Kimberly A. Wilcox, F. Abron Franklin, Shannon K. McWeeney, Daniel N. Streblow, Guang Fan, and Donna E. Hansel*

ABSTRACT: Multiple studies worldwide have confirmed that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA can be detected in wastewater. However, there is a lack of data directly comparing the wastewater SARS-CoV-2 RNA concentration with the prevalence of coronavirus disease 2019 (COVID-19) in individuals living in sewershed areas. Here, we correlate wastewater SARS-CoV-2 signals with SARS-CoV-2 positivity rates in symptomatic and asymptomatic individuals and compare positivity rates in two underserved communities in Portland, Oregon to those reported in greater Multnomah County. 403 individuals were recruited via two COVID-19 testing sites over a period of 16 weeks. The weekly SARS-CoV-2 positivity rate in our cohort ranged from 0 to 21.7% and trended higher than symptomatic positivity rates reported by Multnomah County (1.9–8.7%). Among the 362 individuals who reported symptom status, 76 were symptomatic and 286 were asymptomatic. COVID-19 was detected in 35 participants: 24 symptomatic, 9 asymptomatic, and 2 of unknown symptomatology. Wastewater testing yielded 0.33–149.9 viral RNA genomic copies/L/person and paralleled community COVID-19 positive test rates. In conclusion, wastewater sampling accurately identified increased SARS-CoV-2 within a community. Importantly, the rate of SARS-CoV-2 positivity in underserved areas is higher than positivity rates within the County as a whole, suggesting a disproportionate burden of SARS-CoV-2 in these communities.

KEYWORDS: SARS-CoV-2 PCR, community testing, wastewater-based epidemiology

INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become one of the greatest global health challenges of the 21st century.1–3 While all age ranges of the population are susceptible to SARS-CoV-2 infection, clinical manifestations of disease have varied and changed over time. Common symptoms include fever, dry cough, loss of taste or smell, and malaise.4–6 Gastrointestinal symptoms such as diarrhea, anorexia, nausea, and vomiting may also occur.7,8 Elderly individuals with co-morbidities are more likely to develop severe respiratory symptoms requiring hospitalization, whereas young people and children tend to develop mild symptoms or are asymptomatic.9,10 Compared to the general population, racial and ethnic minority groups are disproportionately affected by the COVID-19 pandemic due to several biomedical and social factors, including cultural distrust of the medical system, limited access to healthcare, poor living and working conditions, and chronic medical conditions.10 Partnership between clinicians and communities may help reduce the short-term impact of COVID-19 disparities.

One key challenge in addressing the COVID-19 pandemic has been monitoring and predicting disease occurrence in the community. Wastewater-based epidemiology (WBE), a type of environmental infectious disease surveillance, has long been suggested to be a sensitive method to monitor pathogen incidence in this setting.11–13 Infected individuals may shed pathogens into the sewage system via feces, urine, saliva, respiratory secretions, and other body fluids and, therefore, the concentration of pathogen in the sewage system is likely proportional to the number of infected individuals among the...
population in the sewersheds area.\textsuperscript{13} In 2003, when the first SARS outbreak took place in China, SARS-CoV viral RNA was detected in the sewage of hospitals treating SARS patients, although no live virus was isolated and RNA detection was considered most likely due to proper disinfection procedures.\textsuperscript{14} During the current COVID-19 pandemic, interest in WBE was renewed when SARS-CoV-2 RNA was identified in patient feces.\textsuperscript{15} Multiple studies worldwide have since confirmed that SARS-CoV-2 RNA can be detected in wastewater and sludge.\textsuperscript{16−18} In addition, the dynamics of SARS-CoV-2 RNA in wastewater appears to correlate with the dynamics of the reported cases, and the occurrence of SARS-CoV-2 variants of concern (VOCs) can be captured by genomic sequencing.\textsuperscript{19−21} Our own experience with monitoring Oregon communities over a 10-month period has suggested that WBE is a better predictor of COVID-19 community prevalence than routinely reported cases, despite WBE’s inherent variability.\textsuperscript{22}

In the current study, we sought to determine the correlation between wastewater SARS-CoV-2 signals and individual COVID-19 status, as well as compare positivity rates in two underserved communities in Portland, Oregon to those reported in the greater Multnomah County. To execute this study, we established two community-based SARS-CoV-2 testing sites to compare population SARS-CoV-2 infection rates with prospective wastewater signals, with an emphasis on rates of infection in communities disproportionately affected by SARS-CoV-2. We used a saliva-polymerase chain reaction (PCR) test as our primary detection assay. This assay has equivalent viral detection capability in asymptomatic patients and facilitates minimally invasive specimen collection. Symptomatic individuals were also offered the option of a nasopharyngeal swab-PCR test. The study was designed to assess how well wastewater sampling can accurately identify increased SARS-CoV-2 transmission in the communities tested in this study.

\section*{Materials and Methods}

\textbf{Community Subject Recruitment.} This study was approved by the institutional review board (IRB) at Oregon Health & Science University (protocol STUDY00022151). Self-Enhancement, Inc., a community-based organization, established community COVID-19 testing sites in disproportionately affected areas in Northeast (92-159th NE Sandy Blvd, Portland, OR 97230) and Southeast (118-162nd SE Powell Blvd, Portland, OR 97226) Portland. Clean Water Service and Portland Bureau of Environmental Services identified four sewersheds that cover the neighborhoods within the study population.

Two walk-up COVID-19 community testing sites, Prescott Elementary School (site #1) and Imago Dei Eastside Church (site #2), were selected due to close proximity to the sewersheds regions. Weekly community COVID-19 testing was conducted in both sites (11/16/2020−3/8/2021, 16 weeks in site #1, and 11/19/2020−1/14/2021, 9 weeks in site #2). Participants were voluntary and recruited using flyers, Facebook posts, and mass emails sent by Self-Enhancement, Inc., Imago Dei Eastside Church, and Prescott Elementary School. All individuals living or working in communities identified for the study were eligible for testing, including those who were asymptomatic. Every individual presenting at the testing site received detailed information about the study. Participants had the option to receive COVID-19 testing only and decline the study or participate in our study via informed consent. Research participants were asked to complete a survey questionnaire collecting risk factors, demographics, history of exposure to patients infected with SARS-CoV-2, details about the timing of onset of COVID-19 symptoms, and pre-existing medical conditions. Participants were also asked to give yes/no answers to the optional study components, including (1) storage of data and sample in a biorepository and (2) contact in the future for other potential research opportunities. SARS-CoV-2 isolates from consented participants who agreed to the optional study were stored in the biorepository for additional sequencing analysis. Participants were asked to report symptoms over the past month, including documented fever (>38 °C), subjective fever, chills, muscle aches, sore throat, runny nose (not allergy), cough, shortness of breath, headache, nausea or vomiting, and loss of smell or taste. Reporting any one of the symptoms from the list was defined as symptomatic.

The entire cohort was further divided into two sub-cohorts depending on the participants’ zip codes. This allowed us to accurately evaluate the data among the COVID-19 prevalence in the greater Multnomah County versus the wastewater sewersheds areas. The wastewater sub-cohort consisted of participants living or working in one of the nine zip codes within wastewater sewersheds areas: 97213, 97215, 97216, 97218, 97220, 97230, 97233, 97236, and 97266. The Multnomah County sub-cohort consisted of the aforementioned zip codes and the zip codes 97015, 97030, 97045, 97080, 97201, 97202, 97203, 97206, 97208, 97209, 97211, 97212, 97213, 97214, 97217, 97219, 97221, 97222, 97228, 97231, 97232, and 97290. Zip code information was retrieved from \textit{oregonmetro.gov}.

The weekly SARS-CoV-2 test positivity rate in Multnomah County was retrieved from the website of Oregon Health Authority (OHA).\textsuperscript{23} The weekly positivity rate in our cohort was calculated as the number of people tested positive of that week/the total number of participants tested of that week.

\textbf{RNA Extraction, Real-Time PCR, and Next-Generation Sequencing for Saliva and Nasopharyngeal Samples.} Asymptomatic individuals were tested using a saliva-PCR test, whereas symptomatic individuals were offered the option of a nasopharyngeal swab-PCR test. The saliva and nasopharyngeal swab collection and storage, RNA extraction, and real-time PCR have been previously described.\textsuperscript{23} Briefly, RNA was extracted using one of the three different automated machines: Maxwell RSC Instrument (Promega, Madison, MI), MagNA Pure 96 System (Roche Diagnostics, Pleasanton, CA), and KingFisher Flex Purification System (Thermo Fisher Scientific, Waltham, WA). The extracted RNA was tested with either the 2019-nCoV CDC emergency use authorization kit (Integrated DNA Technology, Coralville, IA) or the TaqPath multiplex reverse transcription (RT)-PCR COVID-19 kit (Thermo Fisher Scientific, Waltham, WA). All PCR reactions were performed in a QuantStudio 5 thermocycler (Thermo Fisher Scientific, Waltham, WA) in duplicates. Any specimen with a \(C_T \leq 37\) was considered positive.

To obtain a complete viral genome, next-generation sequencing (NGS) using the Illumina COVIDSeq Test, performed on a NextSeq 550Dx Instrument (Illumina, San Diego, CA), was undertaken on samples that tested positive for SARS-CoV-2 by PCR. The sequence and lineage were analyzed by Illumina DRAGEN COVIDSeq Test Pipeline software.

The phylogenetic tree of the isolate sequences was constructed using the neighbor joining method using Geneious.
### Table 1. Characteristics of the Study Cohort

|                     | entire cohort | wastewater sub-cohort | Multnomah County sub-cohort |
|---------------------|---------------|------------------------|-----------------------------|
| site #1             | (n = 308)     | (n = 84)               | (n = 240)                   |
| age, median [range] | 32.0 [2, 80]  | 28.0 [6, 75]           | 34.0 [2, 80]                |
| sex*, n (%)         |               |                        |                             |
| female              | 175 (58.7%)   | 51 (54.8%)             | 148 (63.5%)                 |
| male                | 117 (39.3%)   | 42 (45.2%)             | 83 (35.6%)                  |
| non-binary, or choose not to identify | 6 (2.0%) | 0 (0%) | 2 (0.9%) |
| race or ethnicity*, n (%) |          |                        |                             |
| black, Afro-Caribbean or African-American | 36 (11.8%) | 11 (11.6%) | 31 (13.0%) |
| Hispanic American or Latino | 34 (11.1%) | 7 (7.4%) | 26 (10.9%) |
| non-Hispanic White or Euro-American | 177 (58.0%) | 45 (47.4%) | 130 (54.4%) |
| East Asian or Asian | 35 (11.4%) | 18 (18.9%) | 32 (13.4%) |
| South Asian or Indian American | 5 (1.6%) | 8 (8.4%) | 6 (2.5%) |
| Middle Eastern or Arab American | 3 (1.0%) | 0 (0%) | 1 (0.4%) |
| Native Hawaiian or Pacific Islander | 2 (0.7%) | 1 (1.0%) | 3 (1.3%) |
| American Indian or Alaskan Native | 6 (2.0%) | 0 (0%) | 4 (1.7%) |
| two or more         | 14 (4.6%) | 5 (5.3%) | 15 (6.3%) |
| declined            | 6 (2.0%) | 2 (2.1%) | 3 (1.3%) |
| symptomatic\d       | 60 (21.6%) | 16 (19.0%) | 39 (18.0%) |
| COVID-19 PCR detected | 23 (7.5%) | 12 (12.6%) | 18 (7.5%) |
| testing via saliva  | 294 (95.5%) | 73 (76.8%) | 218 (90.8%) |

*Sex (n): in the “entire cohort”: site #1 (n = 298), site #2 (n = 93), and total (n = 391); in the “wastewater sub-cohort”: site #1 (n = 172), site #2 (n = 61), and total (n = 233); and in the “Multnomah County sub-cohort”: site #1 (n = 285), site #2 (n = 82), and total (n = 367). Fisher’s exact.

\dRace or ethnicity (n): in the “entire cohort”: site #1 (n = 305), site #2 (n = 95), and total (n = 400); in the “wastewater sub-cohort”: site #1 (n = 178), site #2 (n = 61), and total (n = 239); and in the “Multnomah County sub-cohort”: site #1 (n = 282), site #2 (n = 82), and total (n = 364).

\dSymptom status reported (including symptomatic and asymptomatic) (n): in the “entire cohort”: site #1 (n = 278), site #2 (n = 84), and total (n = 362); in the “wastewater sub-cohort”: site #1 (n = 162), site #2 (n = 55), and total (n = 217); and in the “Multnomah County sub-cohort”: site #1 (n = 259), site #2 (n = 73), and total (n = 332).

Prime 2022.0.1 (https://www.geneious.com). The SARS-CoV2 VOC reference sequences were downloaded from GISAID and NCBI, including B.1.617.1 kappa variant (EPI_ISL_1372093), B.1.617.2 delta variant (EPI_ISL_2131509), USA/21CA/2020 (MN985325), Wuhan SARS-CoV2 (NC_045512), P.1 gamma variant (EPI_ISL_2048814), L.1315 beta variant (EPI_ISL_601443), and B.1.1.529 omicron variant (EPI_ISL_6640916).

Wastewater Neighborhood Viral Load Surveillance. Four manholes located within the sewersheds were identified to sample the desired area. Sewersheds were almost entirely residential with only some commercial properties. No industrial discharge was present in these areas. The most representative sampling method available in our facility included collection of time-paced 24 h composites with aliquots taken every 30 min. Samples were analyzed twice a week. Aliquots of approximately 40 mL were vacuum filtered and concentrated through a cellulose-ester electronegative filter (Millipore, Bedford, MA). After filtration, the membranes were homogenized with glass beads in 1 mL of the DNA/RNA shield (Zymo Research, Irvine, CA) using either a Qiagen TissueLyser (Qiagen, Germantown, MD) or Biospec Mini-Beadbeater 16 (BioSpec Products, Bartlesville, OK) for 2 min. Lysates were collected by centrifugation, and approximately 200–400 μL of lysates was transferred to a 96-well plate. RNA was extracted using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit on a KingFisher automated instrument (Thermo Fisher Scientific, Waltham, MA) and stored at −80 °C until further analysis.

Droplet digital PCR, specifically a QX200 ddPCR system (Bio-Rad, Hercules, CA), was used to quantify the SARS-CoV-2 viral load in wastewater. The 20.0 μL of the water-in-oil emulsion reaction mixture partitioned by the automated droplet generator consisted of 5 μL of RNA, 1 μL of reverse transcriptase, 5 μL of Superscript from the One-Step RT-ddPCR Advanced Kit for Probes, 1 μL of primer/probe sets targeting the viral nucleocapsid gene, and an internal control RNase P from 2019-nCoV CDC ddPCR Triplex Probe Assay. Commercially available synthetic SARS-CoV-2 RNA transcripts containing five gene targets (E, N, ORF1a, and RNA-dependent RNA polymerase) were used as standard to quantify the viral load (Exact Diagnostic, Fort Worth, TX). All samples were tested in duplicate. After thermocycling, the plate was then transferred to the QX200 Droplet Reader and analyzed using QuantaSoft droplet reader software.

The twice weekly result of viral load from each sewershed was averaged each week as the viral gene copies per estimated total population. The estimated population was calculated as the number of residences in the area × 2.5, which we believe that it represents rough estimates of the static population. For the dates when SARS-CoV-2 RNA was undetectable, the RNA value was plotted as 1/2 of the limit of detection (LOD), to represent that the true value is some unknown between 0 and the LOD. 20% of the samples (23 of 115) were below LOD; therefore, the 1/2 LOD substitution is considered appropriate.
Figure 1. Schematic presentation of the study design. The entire cohort was recruited from two COVID-19 community testing sites in disproportionately affected areas in northeast and southeast Portland, OR. Four manholes at selected spots were identified to capture the desired sewershed. The entire cohort is further divided into two subcohorts depending on participants’ zip codes. The wastewater sub-cohort consists of participants living or working in the wastewater sewershed areas. The Multnomah County sub-cohort consists of participants living or working within Multnomah County. The comparison between the wastewater SARS-CoV-2 viral load and general population positivity rates within the corresponding sub-cohort was performed.

Table 2. Characteristics of the Wastewater Sub-cohort

|                        | wastewater sub-cohort | p-value | total (n = 240) |
|------------------------|-----------------------|---------|-----------------|
| age, median (range)    | 35.0 [2, 80]          | 0.157   | 34.0 [2, 80]    |
| sexa, n (%)            |                       |         |                 |
| female                 | 110 (64.0%)           | 0.907   | 148 (63.5%)     |
| male                   | 60 (34.9%)            | 0.553   | 83 (35.6%)      |
| non-binary, or choose not to identify | 2 (1.2%) | 1.00b | 2 (0.9%) |
| race or ethnicityb, n (%) |                    |         |                 |
| black, Afro-Caribbean or African-American | 21 (11.8%) | 0.357 | 31 (13.0%) |
| Hispanic American or Latino | 19 (10.7%) | 0.862 | 26 (10.9%) |
| non-Hispanic White or Euro-American | 101 (56.7%) | 0.213 | 130 (54.4%) |
| East Asian or Asian American | 22 (12.4%) | 0.425 | 32 (13.4%) |
| South Asian or Indian American | 3 (1.7%) | 0.175b  | 6 (2.5%) |
| Middle Eastern or Arab American | 1 (0.6%) | 1.00b | 1 (0.4%) |
| Native Hawaiian or Pacific Islander | 2 (1.1%) | 1.00b | 3 (1.3%) |
| American Indian or Alaskan Native | 4 (2.3%) | 0.575b | 4 (1.7%) |
| two or more            | 10 (5.6%)             | 0.542b  | 15 (6.3%)      |
| declined               | 3 (1.7%)              | 0.572b  | 3 (1.3%)       |
| symptomaticc, n (%)    | 31 (19.1%)            | 0.444   | 39 (18.0%)     |
| COVID-19 PCR detected  | 11 (6.2%)             | 0.258b  | 18 (7.5%)      |
| testing via saliva     | 171 (95.5%)           | <0.0001 | 218 (90.8%)    |

“Sex (n): site #1 (n = 179), site #2 (n = 61), and total (n = 233). Fisher’s exact. “Race or ethnicity (n): site #1 (n = 178), site #2 (n = 61), and total (n = 239). Symptom status reported (including symptomatic and asymptomatic) (n): site #1 (n = 162), site #2 (n = 55), and total (n = 217).”

Geographic Distribution of the Study Cohort. The thematic map showing the distribution of wastewater sewershed areas and community COVID-19 participants was analyzed using ArcGIS software (ArcGIS Pro, version 2.8.4) by Esri (Redlands, CA) and illustrated using the map produced by the U.S. Geological Survey (USGS).

Statistical Analysis. Study data were collected and managed using REDCap electronic data capture tools hosted at Oregon Health & Science University. All data management and analyses were performed in SAS version 9.4 (SAS Inc., Cary, NC). Data were analyzed using descriptive statistics, independent sample t tests, Chi-square tests, and Fisher’s exact where appropriate and noted. All significance testing used a two-sided alpha cut-off of 0.05.

RESULTS

Cohort Characteristics. A total of 403 individuals were recruited into the study, including 308 individuals from site #1 and 95 individuals from site #2 (Table 1). A total of 285 participants living in Multnomah County presented to site #1, of which 179 (63%) lived in the sewershed. A total of 82 Multnomah County residents presented to site #2, of which 61
(74%) lived in the sewershed. Of the total cohort \((n = 403)\), 36 (9%) participants neither lived nor worked in Multnomah County. Work zip codes were provided by 156 participants, of which 58 (37%) participants both lived and worked in the sewershed, 27 (17%) lived in the sewershed but worked in Multnomah County, 26 (17%) lived and worked in Multnomah County, 19 (12%) worked in the sewer shed but lived in Multnomah County, 21 (13%) lived and worked outside of the county, and 5 (3%) worked in the sewershed but lived outside the County. There were slightly more female than male participants (57.8 vs 40.7%). The median age of the entire cohort was 31.0 years old (ranging from 2 to 80 years old). More than half (55.8%, 223) of the participants were non-Hispanic White or European-American, followed by Asian/Asian American (13.3%, 53), Black/African American (11.8%, 47), and Latino/Hispanic American (10.3%, 41). The remaining participants were South Asian/Indian American (3.3%, 13), American Indian (1.5%, 6), Middle Eastern (0.75%, 3), and Native Hawaiian (0.75%, 3). There were 19 participants who claimed two or more races and 8 people declined to release the information.

Among the 362 individuals who reported symptom status, 76 (21.0%) were symptomatic and 286 (79.0%) were asymptomatic. SARS-CoV-2 was detected in 35 (8.7%) participants, of which 24 were symptomatic, 9 were asymptomatic, and 2 were unknown. A total of 31.5% (24/76) of symptomatic participants tested positive, while only 3.1% (9/286) of asymptomatic participants tested positive. Symptom status and SARS-CoV-2 test results were significantly associated \((p < 0.001)\).

We further stratified the cohort into two sub-cohorts based on the reported zip codes (Figure 1). This allowed us to accurately assess and compare the data from the wastewater sewershed areas to the general population in Multnomah County. The wastewater sub-cohort consisted of participants living or working in the selected wastewater micro-sewershed areas. The Multnomah County sub-cohort consisted of participants living or working within Multnomah County.

The wastewater sub-cohort of 240 individuals had a median age of 34.0 (Table 2). Approximately 60% of the participants were female (63.5%, 148), 35% were male (35.6%, 83), and two people chose not to identify. The race and ethnicity distributions were similar to those of the entire cohort, with non-Hispanic White as the predominant group (54.4%, 130) followed by Asian/Asian American (13.4%, 32), Black/African American (13.0%, 31), and Latino/Hispanic American (10.9%, 26). Among the 217 individuals who reported symptom status, 39 (18.0%) were symptomatic and 178 (82.0%) were asymptomatic. SARS-CoV-2 was detected in 18 participants, of which 11 were symptomatic, 6 were asymptomatic, and 1 was unknown.

The Multnomah County sub-cohort consisted of 367 individuals (Table 3), of whom 211 were female and 139 were male (57.5 vs 37.9%, respectively). The race and ethnicity distributions were similar to the entire cohort and wastewater sub-cohort, with non-Hispanic White as the predominant group (56.0%, 204). The numbers of Asian/Asian American and Black/African American groups were very similar to 46 (12.6%) and 45 (12.4%) individuals, respectively. The Latino/Hispanic American group consisted of 37 individuals (10.2%) followed by 19 individuals claiming two or more races (5.2%). Among the 332 individuals who reported symptom status, 69 (20.8%) were symptomatic and 263 (79.2%) were symptomatic.
decreased. The average numbers of in-person visits per day per site was 26 before January 4, 2021 and only 6 after January 4, 2021. Therefore, the 0% positivity rate may not be representative of the actual rate. Community study participation and demand for testing declined after the first COVID-19 vaccine was approved by FDA on December 11, 2020.

The geographic distribution of the wastewater cohort and wastewater sewershed areas is shown in Figure 3. The participants were evenly distributed around the four sewersheds by visual observation. The individuals with positive SARS-CoV-2 results were sporadically located without distinct clusters identified.

Community SARS-CoV-2 Positivity in Underserved Areas Was Higher Than General County-Reported Rates of Symptomatic-Only Individuals. In the next part of our study, we evaluated our Multnomah County sub-cohort with the published Multnomah County positivity rate over the same 16 week period from November 16, 2020 to March 3, 2021 with the goal of determining differences in the general population versus in underserved areas of Portland (Figure 4). The SARS-CoV-2 positivity rate of all of Multnomah County at large varied between 1.9 and 8.7%. Specifically, November 2020 had the highest monthly average positivity rate (8.1%) followed by December 2020 (6.3%), January 2021 (4.7%), and February 2021 (2.4%). In contrast, the positivity rate of SARS-CoV-2 in the study population (Multnomah County sub-cohort) was higher than the general population in November 2020, December 2020, and early January 2021, with the peak rates of 13.2% (95% CI: 2.4–23.9%), 12.5% (95% CI: 3.8–21.2%), and 21.7% (95% CI: 4.9–38.6%), respectively. The positivity rates dropped to 0% after late January 2021, which was also associated with a decline in study site participants (from an average of 26 individuals tested per day per site to only six individuals tested per day per site). While there was no statistically significant difference in the positivity rates between all of Multnomah County and our Multnomah County sub-cohort in November and December ($p$-value = 0.22 and 0.21, respectively), there was a significant difference in the rates in January ($p$-value = 0.01). Importantly, our cohort included both symptomatic and asymptomatic participants, and SARS-CoV-2 were detected in participants regardless of their symptoms.

Field Isolates from Study Participants Were Mainly B.1 Sub-lineages. NGS sequencing was performed on the 37 SARS-CoV-2 positive field isolates from study participants. Sequencing results revealed that most of the variants were the B.1 sub-lineages, which was circulating in the USA (Table 4). Two isolates with UK lineage and three isolates with Northern Italian lineage were identified, suggesting viral spread via international traveling. No VOCs, including B.1.1.7 (alpha variant), B.1.351 (beta variant), B.1.617.2 (delta variant), P.1 (gamma variant), and BA.1 (omicron variant), were found in this population. Phylogenetic analysis revealed that the field isolates are clustered and distant from the VOCs (Figure 5).

DISCUSSION

In this study, we determined the onset, duration, and location of SARS-CoV-2 transmission in communities disproportionately affected by COVID-19 in the Portland Metro area using...
wastewater analysis. Our study suggested that the wastewater sampling accurately identified increased SARS-CoV-2 viral RNA levels in the communities, with underserved community rates lagging behind WBE rates. Importantly, the rate of SARS-CoV-2 positivity in individual samples in underserved areas was higher than general County-reported rates of symptomatic-only individuals, suggesting an even higher rate of spread and disproportionate effect of COVID-19 in these communities.

During the early months of the pandemic, several publications showed the presence of SARS-CoV-2 RNA in wastewater, and these results correlated with reported COVID-
For example, in the Netherlands, qRT-PCR targeting one nucleocapsid gene (N3) demonstrated a detectable signal in sewage 6 days before the first case was reported in Amersfoort. However, the remaining qRT-PCR primer/probe sets N1, N2, and envelope gene (E) were negative. Nevertheless, the concentration of the gene fragments in the wastewater increased from 2.6 to 30 gene copies/mL to up to 790–2200 gene copies/mL when the prevalence of COVID-19 in the tested cities such as Tilburg, Utrecht, Amsterdam, and Den Haag increased at the same time. These data suggest that the wastewater surveillance could be a sensitive tool to monitor the virus circulation but it may be challenging at the very early stage of a pandemic.

Ahmed et al. reported the first confirmed detection of SARS-CoV-2 in untreated wastewater in Australia using various qRT-PCR primer sets and virus concentration methods. As a proof-of-concept study, they were able to successfully detect 1.9–12 copies of SARS-CoV-2 RNA/100 mL wastewater, and the median prevalence of infection was estimated as 0.096% (95% CI: 0.064–0.142) during the 6 day testing period. However, the researchers were unable to extrapolate the clinical prevalence data within the wastewater sewershed areas since the clinical cases were reported to the city-level healthcare networks. Similarly, Wu et al. reported ∼250 copies/mL SARS-CoV-2 in wastewater in Massachusetts, USA. The authors estimated that roughly 5% of all fecal samples in the sewershed area were positive for SARS-CoV-2 during the sampling period, which was much higher than the 0.026% confirmed cases during the sampling period. These discrepancies confirm the complexity of correlating viral quantities in wastewater with community prevalence.

Recently, a significant number of studies have been published that improve confidence in the ability of WBE to

Table 4. Lineages of the Field Isolates

| lineage | no. of isolates | lineage description |
|---------|----------------|--------------------|
| A | 1 | root of the pandemic |
| B.1 | 3 | European lineage, the origin corresponds to the Northern Italian outbreak early in 2020 |
| B.1.1.186 | 1 | USA lineage, Southern USA |
| B.1.1.518 | 3 | USA/Mexico lineage |
| B.1.2 | 11 | USA lineage |
| B.1.201 | 1 | UK lineage |
| B.1.234 | 4 | USA lineage |
| B.1.311 | 1 | USA lineage |
| B.1.395 | 1 | UK lineage |
| B.1.509 | 1 | USA lineage |
| B.1.577 | 1 | USA lineage, formally part of B.1.2 |
| B.1.587 | 4 | USA lineage, mainly in Oregon |
| B.1.595 | 1 | USA lineage, was B.1.2 |
| failed in sequencing | 4 | NA |

*Information derives from https://cov-lineages.org/lineage_list.html.

Figure 5. Phylogenetic tree of the field isolates from this study. The tree was constructed by the neighbor joining method using Geneious Prime 2022.0.1. The first US isolate identified in Washington state (USA/WA1/2020) was marked in blue and used as the root for this tree. The important VOCs were marked in red. The field isolates from this study were marked in black.
assess SARS-CoV-2 transmission in the community. The topics addressed by these studies range from assessment and normalization of SARS-CoV-2 RNA in wastewater to a better understanding of the relationship between RNA load and COVID-19 clinical cases and hospitalization. The unique feature of our study is the evaluation of the relationship between wastewater RNA load and COVID-19 in an underserved community and the importance of elevating visibility of these communities to public health authorities. Additionally, the establishment of research cohorts in these communities, including asymptomatic individuals, offered a unique opportunity to evaluate wastewater surveillance both in the pre-vaccination and post-vaccination era. In our cohort, 82% of participants who opted to complete the symptom survey were asymptomatic. This is important to note for WBE validation because the WBE and individual SARS-CoV-2 positivity remained associated even during asymptomatic transmission. In the current post-vaccination era, vaccinated people often have mild to no symptoms. However, these people will continue to shed virus but are otherwise unlikely to be tested and reported to the public health authorities, highlighting the value of WBE.

To the best of our knowledge, this paper and a paper authored by several of the coauthors are the only reports that provide a direct and quantitative evaluation of the prevalence of COVID-19 with people living in the wastewater sewersheds areas and the SARS-CoV-2 viral RNA concentration in sewage. The suggested correlation we identified is particularly strong during November and December 2020 when community participation was high. After the first Pfizer-BioNTech COVID-19 vaccine was approved by FDA on December 11, 2020, we noted that cohort subject interest shifted from testing to vaccination. Therefore, it was challenging to evaluate the correlation between the cohort positivity rate with wastewater viral load following vaccine deployment.

WBE can be an essential component of a robust surveillance system, providing real-time, comprehensive, and objective data in a scalable and cost-effective manner and has the potential to predict outbreaks in advance of individual testing. The accurate assessment between wastewater signal and community prevalence requires testing as many participants as possible in sewersheds areas. In the current study, the estimated population within the four sewersheds ranged from 3900 to 9100 persons per area. Although we did not anticipate that every individual would volunteer for testing in the community, the number of participants was still lower than we expected. Another limitation of the current study includes the sampling method. Flow proportional sampling at a high frequency would be ideal; however, our sampling method was the most representative one available.

During a pandemic, minority groups are more likely to have communication gaps due to socioeconomic disadvantage, limited English-language proficiency, and medical literacy. Mistrust and unfamiliarity of health systems can exacerbate these gaps. In the SARS-CoV-2 pandemic, black, indigenous, and people of color (BIPOC) bore a disproportionate proportion of disease and mortality likely due to non-biological determinants of transmissibility. BIPOC populations are more likely to be uninsured and experience higher rates of pre-existing underlying health conditions. In Portland, they are also more likely to be in lower-wage public-facing employment. According to the OHA guideline released on April 21, 2020, “the COVID-19 testing is prioritized to impacted population and all frontline workers. If supplies allow, asymptomatic people within the congregate care or group living systems can be considered for testing.” Therefore, at the time of our study, it was nearly impossible for asymptomatic people in the general population to get tested for COVID-19. Our research study provided what may have been the only opportunity for individuals in our study community to be tested, regardless of their symptom status and/or living condition.

The data presented in this paper show that community SARS-CoV-2 positivity in the underserved areas was higher than the general County-reported rates among symptomatic-only individuals. Research suggests that improvements to access to SARS-CoV-2 screening are critical to reducing community transmission, regardless of symptomology. Arguably, the screening of only symptomatic individuals appears detrimental to vulnerable groups who experience higher severe illness and death rates. Furthermore, standard health intervention methods are less successful than strategies adapted for the local context and community narrative. Therefore, continuous partnerships with community-based organizations to foster sustainable and reliable relationships with uniquely impacted populations are necessary to eliminate health disparities.

## CONCLUSIONS

Wastewater surveillance captures the presence of SARS-CoV-2 shed by individuals living in the community. Here, we successfully established a research cohort of SARS-CoV-2-infected and uninfected subjects in underserved communities in Portland, OR. The association between wastewater SARS-CoV-2 signals and positivity rates in the cohort was established by weekly testing. Moreover, the rate of SARS-CoV-2 positivity in underserved areas was higher than positivity rates within the County as a whole, suggesting a disproportionate burden of SARS-CoV-2 in these communities.

## AUTHOR INFORMATION

### Corresponding Author

Donna E. Hansel — Department of Pathology & Laboratory Medicine, Oregon Health & Science University, Portland, Oregon 97239, United States; orcid.org/0000-0001-7860-4881; Phone: (503)-494-8276; Email: hansel@ohsu.edu; Fax: (503) 494-2025

### Authors

Zhengchun Lu — Department of Pathology & Laboratory Medicine, Oregon Health & Science University, Portland, Oregon 97239, United States; orcid.org/0000-0003-4165-1507

Amanda E. Brunton — School of Public Health, Oregon Health & Science University—Portland State University, Portland, Oregon 97239, United States

Macedeh Mohebnasab — Department of Pathology & Laboratory Medicine, Oregon Health & Science University, Portland, Oregon 97239, United States

Anthony Deloney — Self Enhancement, Inc., Portland, Oregon 97227, United States

Kenneth J. Williamson — Department of Research and Innovation, Clean Water Services, Hillsboro, Oregon 97123, United States

Blythe A. Layton — Department of Research and Innovation, Clean Water Services, Hillsboro, Oregon 97123, United States
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