Background & Objectives

- Throughout the COVID-19 pandemic, a leading indicator of the presence and level of SARS-CoV-2 infection within a community has been the presence of viral RNA in wastewater.
- Fecal shedding of the virus by infected individuals can occur prior to the presentation of symptoms.
- This surveillance capability is an important tool for public health, indicating the presence of the virus in a consistent and uniform measurement.
- Changes in viral copy load found in wastewater samples over time can indicate infection level changes in a community prior to testing of individuals by nasal swab.
- Wastewater surveillance can also inform public policy decisions regarding the allocation of testing and treatment resources, in preparation for a potential outbreak within a community.
- Whole genome sequencing can be implemented to determine the proportions of known variants present within a sample. This is not currently being done routinely in New Jersey or nationally.
- Wastewater testing has the potential to monitor for additional infectious diseases, providing the New Jersey Department of Health with additional resources for disease surveillance.

Goals

- Implement a pilot program to establish a workflow for testing wastewater for the presence of SARS-CoV-2
- Track changes in SARS-CoV-2 levels and variant of concern proportions over time at participating wastewater treatment plants

Background & Objectives

- Collection sites were selected based on criteria set by the FDA; sites were recruited on a volunteer basis to support method research efforts, but also have surveillance data reported.
- Samples were collected by staff at the wastewater treatment plants and transported to the New Jersey Public Health and Environmental Laboratories (PHEL).
- Samples were stored at 3-6°C for a maximum of 1-3 days prior to processing.
- Samples collected in Jan. & Feb. were frozen at -80°C until processing.
- One-liter wastewater samples were provided twice weekly, beginning in January 2022 (data shown through April 28, 2022).

Methods: Sample Collection & Metadata

- Collection from the 1 Liter collected, 20 mL of wastewater was pipetted into a 50 mL conical tube
- RNA Extraction using Qiagen AllPrep PowerVir Viral DNA/RNA Kit was used for total nucleic acid extraction

Methods: Laboratory Workflow

- Viral load was measured by Quantitative PCR for Detection of SARS-CoV-2 RNA was detected, whole genome sequencing was conducted on the sample
- Whole Genome Sequencing
- WGS was conducted using the Illumina S一篇or MiSeq, with ARTIC templates
- QC data averaged, per facility, for all samples with SARS-CoV-2 reads detected

Results: SARS-CoV-2 Detection

- SARS-CoV-2 Positivity Rate in Wastewater from qPCR

Table 2: Sequence Quality Control

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Average number of total reads</th>
<th>Average read length</th>
<th>Average read quality</th>
<th>% reads that align to SARS-CoV-2</th>
<th>% 0X SARS-CoV-2 genome coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NJDOH-1</td>
<td>161,815</td>
<td>151</td>
<td>35.4</td>
<td>1.04</td>
<td>85.4</td>
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<tr>
<td>NJDOH-2</td>
<td>172,423</td>
<td>151</td>
<td>35.2</td>
<td>3.19</td>
<td>89.9</td>
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</tbody>
</table>

Results: Whole Genome Sequencing

- Table 3: SARS-CoV-2 Variant Analysis

<table>
<thead>
<tr>
<th>Site ID</th>
<th>Date</th>
<th>Cycle threshold Value (N gene)</th>
<th>Polymorphisms</th>
<th>Number</th>
<th>Type</th>
<th>Parental lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NJDOH-1</td>
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<td>2</td>
<td>1 Synonymous SNP</td>
<td>Alpha</td>
<td>-</td>
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<td>35.04</td>
<td>9</td>
<td>1 Non-synonymous SNP</td>
<td>Beta</td>
<td>-</td>
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<td>04/14/22</td>
<td>35.58</td>
<td>2</td>
<td>1 Synonymous SNP</td>
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<td>3</td>
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<td>Beta</td>
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<td>1 Non-synonymous SNP</td>
<td>Beta</td>
<td>-</td>
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<tr>
<td>NJDOH-2</td>
<td>04/07/22</td>
<td>undetected</td>
<td>18</td>
<td>12 Non-synonymous SNP</td>
<td>Beta</td>
<td>-</td>
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</tbody>
</table>

Conclusions and Future Directions

- Establishment of workflow and validated protocols (including site selection, sample collection, testing, and reporting) for detection of SARS-CoV-2 in wastewater collected from wastewater treatment plants.
- Using wastewater samples, whole genome sequencing reveals the capability to determine the variants of concern present within a community.

Future Directions

- Test wastewater testing workflow for high throughput testing to allow for larger number of weekly samples and improved detection resolution; Institute long-term wastewater testing program for the New Jersey Public Health and Environmental Laboratories.
- Identify additional sites throughout the state of New Jersey for representative sampling of large populations.
- Explore additional infectious agents that can be monitored in wastewater and track the change in their level over time and across geography of the state of New Jersey.