Background

Wastewater surveillance adds a useful layer of monitoring community levels of SARS-CoV-2. Due to the shift to self-testing and decreased clinical testing, reported COVID-19 case counts are lower than the actual COVID-19 case counts. Using wastewater surveillance data paired with the monitoring of COVID-19 case counts, hospitalizations, and fatalities, this allows for a more complete understanding of disease patterns. When trends are similar across these measures, confidence in the accuracy of those trends increases.

Advantages of monitoring wastewater concentrations of SARS-CoV-2:

- **Wastewater based epidemiology:**
  - Helpful when paired with clinical data since SARS-CoV-2 concentrations in sewage are positively correlated with COVID-19 case counts.

- **Early warning for emerging outbreaks:**
  - Infected people begin shedding 2 - 3 days before onset of symptoms.
  - SARS-CoV-2 is shed in feces by both individuals with asymptomatic and symptomatic COVID-19 infections.

Possible limitations of these data to consider:

- We cannot precisely predict case counts with the detection of concentrations of SARS-CoV-2 in wastewater.
- Wastewater surveillance might not capture low levels of infection in a community.
- Some communities and/or facilities are not connected to a Wastewater Treatment Plant (WWTP).
- Inhibitors could be present in wastewater that impacts the detection of SARS-CoV-2. Inhibition assessments are in place to ensure RNA quantification methods and viral recovery are performing as expected.

Interpretation of Trends

Not all peaks and surges in concentrations will correlate with a community-wide increase in cases. Wastewater is a highly variable mixture where concentrations of all pathogens like SARS-CoV-2 may vary based upon time of collection, or collection methods of sewage. For example, concentrations can vary on whether the sample was a grab sample (only captures at one point of time) or a 24-hour composite sample (more longitudinal variability over time). If an upward trend is observed in the data, this might represent an increase in cases that has yet to be confirmed through case-based surveillance. Additional data is required to confirm whether this trend will persist.

SARS-CoV-2 Variants in Sewage

SARS-CoV-2 is a constantly evolving virus. The detection of SARS-CoV-2 variants in wastewater is another useful layer of surveillance since some variants spread more rapidly than others. Information on dominant or new variants in a community assists in public health response. Additionally, in some cases, variants have been detected in wastewater prior to detection in clinical samples.
Wastewater Surveillance for the State of Hawaii

This report contains results for the SARS-CoV-2 surveillance of sewage in collaboration with the National Wastewater Surveillance System (NWSS). A total of 15 WWTP from the State of Hawaii are participating in this surveillance. Samples are collected weekly and analyzed by Biobot Analytics. Concentrations in this document are reported as SARS-CoV-2 copies per nanoliter of wastewater.

For visualization and interpretation of trends, this report includes regression lines to help visualize possible changes in SARS-CoV-2 concentrations in sewage and COVID-19 case counts over time. For example, if the constant trend in SARS-CoV-2 concentrations is decreasing, we will likely observe a similar decrease in COVID-19 cases.

This report also includes information on the dominant variants in wastewater through Whole Genome Sequencing (WGS). WGS is done with the use of Illumina instruments through Biobot’s trusted sequencing partners. Data are reported in relative abundance, which is the proportion of SARS-CoV-2 variants that are detected. For example, if BA.5 (parent Omicron lineage) is the highest, this means that BA.5 is the dominant variant relative to all other SARS-CoV-2 variants in the wastewater. These WGS data do not include information on the relative abundance of other pathogens present in wastewater.

Due to the variability of wastewater and presence of inhibitors, concentrations reported have been normalized by flow and population served for each WWTP. Excessive rainfall and changes in water use can impact concentrations of SARS-CoV-2 in sewage over time. The use of a fecal indicator control (Pepper Mild Mottle Virus - PMMoV) helps account for any changes in human waste input in wastewater over time. For more information on how wastewater surveillance works, refer to the resources at the end of the report. At the Hawaii Department of Health’s State Lab, we are in the process of validating our own protocols for processing wastewater on-site. This report includes samples collected from 10/1/2022 to 12/20/2022. Report print date: 01/03/2023.

### Metadata for participating WWTP by county

<table>
<thead>
<tr>
<th>County</th>
<th>Number of WWTP</th>
<th>Total Population Served</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honolulu</td>
<td>5</td>
<td>1,180,536</td>
</tr>
<tr>
<td>Maui</td>
<td>3</td>
<td>160,000</td>
</tr>
<tr>
<td>Hawaii</td>
<td>3</td>
<td>37,850</td>
</tr>
<tr>
<td>Kauai</td>
<td>4</td>
<td>18,000</td>
</tr>
</tbody>
</table>
Figure 1. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/nL) and (B) case counts for the state of Hawaii by each county. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: All counties are observing an increasing trend in raw sewage concentrations of SARS-CoV-2. Since the last report, there was a rise in the COVID-19 positivity rate and cases across the state.
SARS-CoV-2 Variants in Wastewater

Figure 2. Stacked barplot demonstrating the relative abundance of (A) SARS-CoV-2 aggregated lineages and (B) all SARS-CoV-2 lineages detected in wastewater across all counties in the state of Hawaii from samples collected in November - December 2022.

Notes: Among the SARS-CoV-2 lineages detected, BA.5*, BQ.1.1* and BQ.1* have the highest relative abundance across counties, followed by BA.2*, XBB*, BF.11*, BN.1* BA.2.75*, XBD, and XBE which are all parent or sublineages of Omicron. Emerging variants being monitored include BA.2.75*, XBB.*, and BN.1* lineages. XBB.1.5 has not been detected in clinical samples across the state but was found in Honolulu County wastewater. For a more detailed description of WGS and information on clinical SARS-CoV-2 variants in the State of Hawaii, refer to the State of Hawaii SARS-CoV-2 Sequencing and Variant Report and the resources at the end of this report.
Resources

  - How it works: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/resources/how-wws-works.html
  - Data Reporting: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/data-reporting-analytics.html
  - Data: https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance

- Biobot Analytics: https://biobot.io/
  - Data: https://biobot.io/data/
  - WGS: https://biobot.io/covid19-variants-report-notes/

- SARS-CoV-2 Variant Classifications:
  - https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html#:~:text=SARS%2DCoV%2D2%20has%20many,contain%20one%20or%20more%20mutations.

- More about wastewater surveillance for SARS-CoV-2:
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8416286/
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7583624/
  - https://www.nature.com/articles/s41586-022-04980-y