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Ohio Coronavirus Wastewater Monitoring Network: implementation of statewide monitoring for protecting public health

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Supplemental Digital Content: Table S1 (Summary of the different methods currently used in the OCWMN) and Figure S1 showing Ohio's 8 hospital preparedness regions are provided as a supplement.

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Abstract

Context: Prior to the COVID-19 pandemic, wastewater influent monitoring for tracking disease burden in sewered communities was not performed in Ohio, and this field was only on the periphery of the state academic research community.

Program: Due to the urgency of the pandemic and extensive state level support for this new technology to detect levels of community infection to aid in public health response, the Ohio Water Resources Center established relationships and support of various stakeholders. This enabled Ohio to develop a statewide wastewater SARS-CoV-2 monitoring network in two months starting in July 2020.

Implementation: The current Ohio Coronavirus Wastewater Monitoring network (OCWMN) monitors over 70 unique locations twice per week, and publicly available data are updated weekly on the public dashboard.

Evaluation: This manuscript describes the process and decisions that were made during network initiation, the network progression, and data applications, which can inform ongoing and future pandemic response and wastewater monitoring.

Discussion: Overall, OCWMN established wastewater monitoring infrastructure and provided a useful tool for public health professionals responding to the pandemic.

Keywords

Wastewater-based epidemiology; early warning; public health response; COVID-19 variants; wastewater surveillance

Context

Wastewater surveillance can provide health and behavior information about communities through detection and interpretation of various markers in wastewater. Few long-term wastewater surveillance initiatives have been established worldwide prior 2019, with most focused-on tracking pharmaceuticals and illicit drugs. [1] Environmental surveillance, including wastewater surveillance, has been used for decades to monitor poliovirus transmission as a part of the Global Polio Eradication Initiative. [2,3]

Early in the COVID-19 pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA was detected in stool specimens of hospitalized patients. [4] Soon after, viral RNA was observed in untreated wastewater in quantifiable amounts worldwide. [5–7] The virus is primarily shed into human waste by the upper respiratory and gastrointestinal systems, although the quantity and importance of these routes are unclear. [8] Since the pandemic onset, wastewater surveillance has been implemented throughout the world as a tool to monitor trends in community levels of COVID-19. [2,9–11,] Wastewater monitoring can be less invasive and more timely than other conventional clinical-based health surveillance approaches and represents all the individuals in the community that are connected to the monitored sewer system. This passive, quantitative monitoring of wastewater is unaffected by underreporting biases inherent with clinical data due to limited access to tests, symptom-based test seeking behavior, and self-diagnosis with at home tests. Furthermore, wastewater monitoring captures asymptomatic, pre-symptomatic, and symptomatic cases. [1,12] An added value of wastewater monitoring lies in its application as an early warning tool for emergence/reemergence of SARS-CoV-2 circulating in a community, since it could provide lead time before infections are identified by other means. [9,13–16]

In May 2020, Ohio's Governor Mike DeWine tasked State Agencies – specifically the Ohio Department of Health (ODH) and the Ohio Environmental Protection Agency (Ohio EPA) with implementing statewide wastewater SARS-CoV-2 monitoring. The goals of the monitoring were to evaluate the trends in amount of SARS-CoV-2 gene copies as a leading indicator of disease occurrence in a community and inform localized community interventions to help limit the spread of disease.

Approach and Evaluation Strategy

The ODH and Ohio EPA decided to leverage local experts and partners and approached state universities to serve as testing laboratories. The Ohio Water Resources Center (Ohio WRC), a federally authorized and state-designated Water Resources Research Institute housed at the Ohio State University (OSU) in the Civil, Environmental and Geodetic Engineering Department, helped to rapidly build and manage this statewide wastewater monitoring network, referred to here as the Ohio Coronavirus Wastewater Monitoring Network (OCWMN). U.S. EPA's, Office of Research and Development (ORD) also provided technical expertise and testing capacity at its Cincinnati, Ohio facilities.

In the following article we will describe our approach to rapidly develop statewide wastewater monitoring for novel pathogen and efforts to interpret and apply the monitoring results.

Leadership and Structure of the OCWMN

Since its inception, the OCWMN was designed as a distributed network with many partners statewide. In June 2020, during the network's initiation, the three main issues in using untreated wastewater to monitor SARS-CoV-2 transmission in communities were: (i) lack of standard methods/procedures for quantifying SARS-CoV-2 RNA in the wastewater; (ii) limited resources and knowledge on application of wastewater monitoring results to inform public health decisions; and (iii) uncertainty regarding virus fecal shedding per infected person. The network leaders established specialized working groups, each with 8 −15 members, to discuss various areas of the network development and make decisions based on the best scientific knowledge at the time. The groups and their organizational participants are displayed in Figure 1. All groups, except the sequencing group, were established in June 2020. The sequencing group was added to the network in February 2021.

The purpose of the leadership group was to develop statewide wastewater monitoring for SARS-CoV-2 that provides additional data to public health officials to prepare for and mitigate COVID-19 spread. The group was composed of project leaders at Ohio WRC, Ohio EPA, ODH and US EPA and of leaders of the other groups in the network. Later on, the leadership group added representatives from the Ohio Department of Agriculture and the Ohio Department of Corrections and Rehabilitation. The goal of the group was to coordinate the network and provide needed communication between the different project partners, while also acting as a main contact body for interaction outside of the network with state, regional, and national partners. The group met weekly in the first year of the network establishment.

In order to establish a monitoring network as quickly as possible, the group (i) recruited a network of research laboratories; (ii) allowed for processing and PCR method diversity to leverage lab equipment and expertise while ensuring continuous optimization and innovation of methods; (iii) created several working groups of experts to advise the leadership about various aspects of the project; and (iv) solicited both research and practical information from experts nationwide and internationally.

Site Selection Group

The main purpose of the site selection group was to identify and prioritize locations for monitoring. The group was composed of wastewater treatment experts from OSU's Civil, Environmental and Geodetic Engineering Department, Ohio EPA and US EPA together with ODH public health professionals. The goal of the group was to select sites that meet the specified criteria outlined below and recruit wastewater utilities. The group met frequently during the project initiation and later only on as needed basis.

The monitoring site consideration at the beginning of the network initiation was affected by the organization of the public health system in Ohio and the timing of the network development. The public health system in Ohio is comprised of 8 hospital preparedness regions (see supplement Figure S1) [17] and 113 local health departments. During late spring 2020, when discussions of the wastewater monitoring network started, Ohio was reopening after a month in lockdown and ODH released a county-based COVID-19 Public Health Advisory System. [18] In line with the county-based approach to public health decisions in Ohio, it was decided that the sites should target the whole municipality/sewer area scale and samples should be collected at the influent to the wastewater treatment plants (WWTPs).

The main criteria for site selection, as developed by the Site Selection Group, was: (i) multiple municipalities of differing sizes in every one of Ohio's hospital preparedness regions; (ii) community vulnerability based on May 2020, ODH Health360 Coronavirus Response Management Analytics report (vulnerability was defined as census tract with high prevalence of COVID-19 underlying conditions, such as age and health; high concentration of low-wage essential workers; households that have difficulty with social distancing; households without capacity to weather long-term social and economic upheaval;

and households without necessary resources located in proximity, such as hospitals, transportation); (iii) wastewater treatment facility flow volume and population served; and (iv) willingness to participate.

Once sites were selected, the individual wastewater treatment facility superintendents or directors were contacted and invited to participate in the network. Meetings were held with the facility leaders, staff and sometimes city representatives of the participating facilities to discuss the purpose of the monitoring, the known science to support the public health relevance of the monitoring, how the results would be used and applied, and details requested of the facility for participation (e.g., site information, days of sampling, sample collection and handling procedures). During the onboarding process, ODH reached out to the local health district in the utility jurisdiction and informed them about the utility participation and the monitoring goals.

OCWMN has been collecting wastewater from 77 distinct wastewater treatment facilities that serve a population of approximately 6,250,000, which represents 54% of Ohio's population. Most of the monitored sites were recruited between July and October 2020. The locations of facilities currently involved in monitoring are displayed in the map below (Figure 2). The facilities participate voluntarily in the network and have not been compensated. Therefore, the network emphasizes placing minimum additional burden on facilities. The participating wastewater treatment plants are provided with needed supplies and pre-paid shipping labels, or preferably courier services are arranged to pick up samples and drop off new supplies. Sampling at most facilities is performed twice a week (usually Sunday/Monday composite and Tuesday/Wednesday composite), since it was shown for trend analyses that a minimum of two non-consecutive samples per week are needed. [19] The program leverages the influent sampling methods used at the participating facilities for NPDES permits and 97% of samples are collected as 24hr time or flow composite samples. After collection, samples were kept in fridge at the facility or packaged with ice (maximum 4 hours) until sample pick up by courier or drop off at shipping location. Sample delivery/shipment is a crucial part of the monitoring network, since samples that are not maintained at refrigerated temperatures are considered compromised and not analyzed if the temperature at receipt is 15°C or higher.

About 21% of the contacted facilities declined to participate in the network due to additional burden on the facility or reluctance expressed by the municipality leadership. In these cases, the site selection group attempted to recruit facilities in the same region with similar community size. This lack of participation did not impact our ability to inform public health, since we recruited a number of diverse communities in each region. Furthermore, local health district personnel reported using neighboring county data in the absence of wastewater monitoring in their county.

Lab Analytical Methods Group

The main purpose of the lab analytical methods group was to provide advice to OCWMN leadership about processing and quantification methods and procedures for SARS-CoV-2 gene fragments quantitative measurements in wastewater. The group was composed of microbiology and molecular biology experts from universities, state and federal agencies and commercial laboratories. The goal of the group was to share and discuss analytical methodologies and quality assurance (QA) and quality control (QC) procedures. The group met once a week during the first year of the monitoring and once every two weeks during the second year of the monitoring.

Based on the State needs and the conditions during the network development, the analytical resource group decided on a set of QA/QC procedures that each participating laboratory must perform. The general procedures and QA/QC process involved: (i) two to three-day turn-around time from sample receipt to data reported to Ohio WRC; (ii) no sample pasteurization, which has been supported by research[12,20] reporting 50%– 90% loss of RNA signal after sample pasteurization; (iii) sample holding temperatures maintained at 2–8°C before processing. Published work confirmed that a sample holding temperature at 4– 5°C was acceptable for up to 5 days without a substantial loss of SARS-CoV-2 RNA[12]; (iv) wastewater samples were processed in technical duplicates; (v) a coronavirus surrogate (e.g., OC43, bovine coronavirus (BCoV)) is spiked into wastewater prior to processing to calculate recovery efficiency of processing methods; (vi) assessment of PCR inhibition; (vii) measurement of human fecal indicator (e.g., pepper mild mottle virus (PMMoV) and/or crAssphage) for potential fecal strength normalization and internal control; (viii) report limit of detection (LOD), which is a combination of the instrument detection limit (concentration that the instrument can reliably distinguish from the background) and sample concentration factor (depending on the methodology).

The list of the laboratories that participated in the network during July 2020 – December 2021 and the various processing, nucleic acid extraction and PCR methods used are summarized in the Supplement - Table S1. Using multiple academy laboratories enabled us to leverage state expertise and rapidly build laboratory analytical capacity for wastewater monitoring statewide (some laboratories were already monitoring wastewater at the facilities nearby). At the time of the network establishment, there were reports indicating variability in results among methods, but the variability was not well defined. Two of the network laboratories participated in a larger Water Research Foundation interlaboratory comparison study[21] that demonstrated a range of 2.3 $\log_{10}$ in recovery adjusted SARS-CoV-2 genome copies/L values among 36 standard operating procedures. To understand the variability in measurements produced by the Ohio's network laboratories, bi-monthly split-sample trials were conducted, whereby each lab received and analyzed an aliquot of a composite sample with unknown quantity of SARS-CoV-2 RNA. From the point of network initiation until December 2021, 8 split-sample trials were performed. In general, the average N2 gene fragments/L spanned 1.5 log10 among the laboratories in the network during each trial (range 0.7 – 2.2 $\log_{10}$).[22] Like other studies, some network laboratories observed differences between N1 and N2 gene fragment copies/L, while other laboratories did not see differences between these two N markers. [12,15,23–27]

In July 2022, the OCWMN transitioned the sample analyses from the multiple research laboratories to ODH Public Health Laboratory (PHL). Although using multiple laboratories provided many advantages initially (Table 1), with more knowledge about the impact of the various analytical procedures and inter lab variability led to decision to use a single laboratory.

Statistics and Modelling Group

The main purpose of the statistics and modeling group was to help with wastewater data interpretation and application. The group was composed of biostatistics and risk assessment and modelling experts from OSU's College of Public health, US EPA and ODH epidemiologists. The goal of the group was to (i) develop methods to evaluate the trends in gene copy concentrations at each location, and (ii) develop a model to describe disease prevalence in the communities. The group met weekly.

Analyses performed by partners of OWMN using Ohio's data indicated that the monitored sites showed significant correlation between SARS-CoV-2 concentrations in wastewater and new case counts in monitored communities. [14,28,29]

For direct public health application, several statistical methods were evaluated for trend analysis using the flow normalized data, including control charts, moving averages and regression analysis. The influent wastewater flow values were provided by the participating wastewater treatment facilities during sample submission and represented the flow volume over the sampling period (million gallons per day). The SARS-CoV-2 load was calculated using the average SARS-CoV-2 gene copies/liter in a sample and multiplying it by the influent flow during sampling using equation below:

$$Million \quad gene \quad copies \quad per \quad day = \quad ((Gene \quad copies / L)(Flow \quad (L / d)))/1,000,000$$

,where L is liters and d is day

Initially a simple tool was developed to evaluate the increase or decrease of the most recent measurements. This tool was based on communication with the Utah Department of Environmental Quality SARS-CoV-2 Sewage Monitoring team (9/1/2020). Here, the four most recent data points were evaluated, by comparing the average of the last two results with the average of the previous two results, and percent difference was calculated. If the newest data average was at least 50% lower or higher than the earlier average, the trend was indicated to be decreasing or increasing, respectively.[30]

For more rigorous trend analyses, additional approaches were suggested and tested on the wastewater gene copy data: (i) Control charts (with 95% upper bound) and (ii) Linear regression. Currently the network utilizes 5-point (2.5 weeks) regression analyses on logarithmically transformed flow normalized data to determine the trend of wastewater SARS-CoV-2 concentrations. Detailed description of the analyses can be found on the OCWMN website.

Various modelling approaches were discussed in the statistics and modelling group meetings and two common models[5, 31] were compared and modified. The team developed a mechanistic model based on a generalized mass balance of SARS-CoV-2 input into the sewer system to translate gene copies concentrations measured in wastewater into a defined number of shedders in the catchment area (publication in preparation). A Spearman-rho sensitivity analysis was conducted on the model and the most impactful parameter on the model uncertainty was the concentration of SARS-CoV-2 per gram of human feces. Common obstacles to modelling the number of infected individuals contributing to wastewater in the various catchment areas are described elsewhere.[32]

Sequencing Group

The main purpose of the sequencing group was to identify SARS-CoV-2 variants in wastewater samples. The group was composed of molecular biology and bioinformatics experts from OSU, US EPA and Battelle Memorial Institute together with ODH public health professionals. The goal of the group was to select and optimize sequencing methods for wastewater samples, discuss best bioinformatic pipelines for sequencing data processing and data interpretation and visualization for use at ODH. The group met frequently during the sequencing initiation.

OCWMN started sequencing samples in January 2021 to track the first SARS-CoV-2 Variant of Concern (VOC), Alpha, as it spread through the state. To complete this analysis the network leveraged three sequencing laboratories: US EPA ORD, OSU Infectious Diseases Institute and Battelle Health Outcomes and Biotechnologies Genomics Laboratory.

Starting in April 2021, processed samples from wastewater collection sites were sequenced biweekly, and since June 2021 sites with sufficient concentrations (RT-qPCR CT levels lower than 36 or at least 3 positive RT-ddPCR droplets) have been sequenced on a weekly basis. All the laboratories performed whole SARS-CoV-2 genome tiled amplicon sequencing (short read sequencing) as this method was hypothesized to be the most generalizable between samples given the heterogeneity of sample processing and RNA extraction methods used in the network. Laboratories used either the slightly modified SNAP SARS-CoV-2 v2 Kit (Swift Biosciences, currently sold as xGen™ SARS-CoV-2 Amp Panel by Integrated DNA Technologies) or ARTIC PCR and a modified COVIDSeq (Illumina, San Diego, CA) protocol. Sequencing was performed on Illumina platforms available to each individual laboratory (HiSeq or NextSeq550 and NextSeq2000). Sequence analysis pipelines were developed independently by each lab, in part due to limitations related to differences in computing resources available to each group. As these pipelines were developed[33], relevant parameter settings (i.e., filtering thresholds) were discussed and standardized among groups, and common datasets were shared and analyzed to help ensure the differing data analysis methods produced similar outputs. Data were reported by each group in the form of the number of reads containing the reference allele (Wuhan reference genome) and the alternative allele associated with amino acid changes for a VOC, or Variant of Interest. Other unique detected amino acid changes were reported as well to allow for retrospective analyses if new variants emerge.

The Alpha variant was Ohio’s dominant variant starting April 2021, as confirmed by sequencing data in both wastewater and clinical cases. Subsequently, a transition to the Delta variant was observed during the summer 2021 and the amino acid changes associated with this variant were dominant thereafter. However, wastewater samples from December 5th, 2021, showed first evidence of the Omicron variant in one Columbus WWTP and in two Cleveland WWTPs. Concurrently, the first Omicron clinical cases in Ohio were reported from two people in Columbus tested on December 7th and one person in Cleveland tested on December 6th.

Results

Since the network initiation, the main goal of the monitoring was to provide early warning of COVID-19 community spread when the numbers of reported SARS-CoV-2 cases are low or close to zero in a community[34], as was the case during the initiation of this network. The other important goal was to provide a supplemental independent tool for communities and public health officials to track the trends of the disease in their communities to inform their decision making and actions. The result inferences were focused on trends at each of the monitored sewersheds and comparison between sewersheds was avoided due to a lack of methodological consistency between groups and a limited understanding of how to compare data from sewersheds with different characteristics.

Retrospective analyses confirmed that SARS-CoV-2 wastewater concentrations were significantly correlated with new cases in most monitored communities and that wastewater concentration was leading indicator of case increase (3 days on average). The wastewater results are important public health information both when cases are consistent or not consistent with the wastewater SARS-CoV-2 signal. [35]

If an increase in SARS-CoV-2 gene copies/L or million gene copies/day was observed in sewage from a monitored community, ODH (i) notified the local health district and WWTPs; (ii) provided information on how to interpret the data and link to a message toolkit (published on OWMNC website[30]); (iii) notified the state testing team, contact tracing and vaccination teams to offer assistance. ODH did not provide alerts when wastewater concentration was decreasing, since the virus can be shed via feces by infected people considerable amount of time after symptoms onset.

About 1,500 notifications about SARS-CoV-2 increases in wastewater have been sent to local health districts and WWTPs since network inception. Although detailed tracking on how these notifications were used in individual communities are missing, a retrospective survey is in preparation to better understand the use of the wastewater information.

From the information that we collected informally in 2020 and 2021, local communities most often: (i) asked for state resources (such as contact tracing/testing teams support); (ii) released public notification emphasizing caution and safe behavior in various formats including press releases, local news, Facebook posts, and even billboards; and (iii) notified local leaders and other stakeholders (hospitals, schools). Since increases in SARS-CoV-2 wastewater concentrations often corresponded or were followed by case increases and local health districts were encouraged to interpret wastewater monitoring data in context of other surveillance data, it is unclear if these actions were solely based on wastewater alerts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Implications for Policy & Practice

- Building statewide monitoring network in a short time frame is an enormous challenge that requires considerable collaboration, resources, and time. Maintaining the established networks will be beneficial for future disease outbreaks.
- During emergencies, monitoring networks have to be built with flexibility and resiliency in mind, since many decisions are made immediately and with limited available information and practices that evolve later as science develops.
- In future wastewater monitoring programs, developing standardized protocols for specific wastewater targets (influenza, antibiotic resistance etc.) and general targets (such as viral RNA metagenomic sequencing) will improve the general usability of the results.
- Due to the relative novelty of the wastewater monitoring data for the public health infectious disease community, development of communication resources and simplification of data interpretation is necessary.
- Creating stronger relationships among the different stakeholders as well as educating the general public about the advantages of wastewater data for communities and developing ethical guidelines is crucial for further success of wastewater monitoring networks.

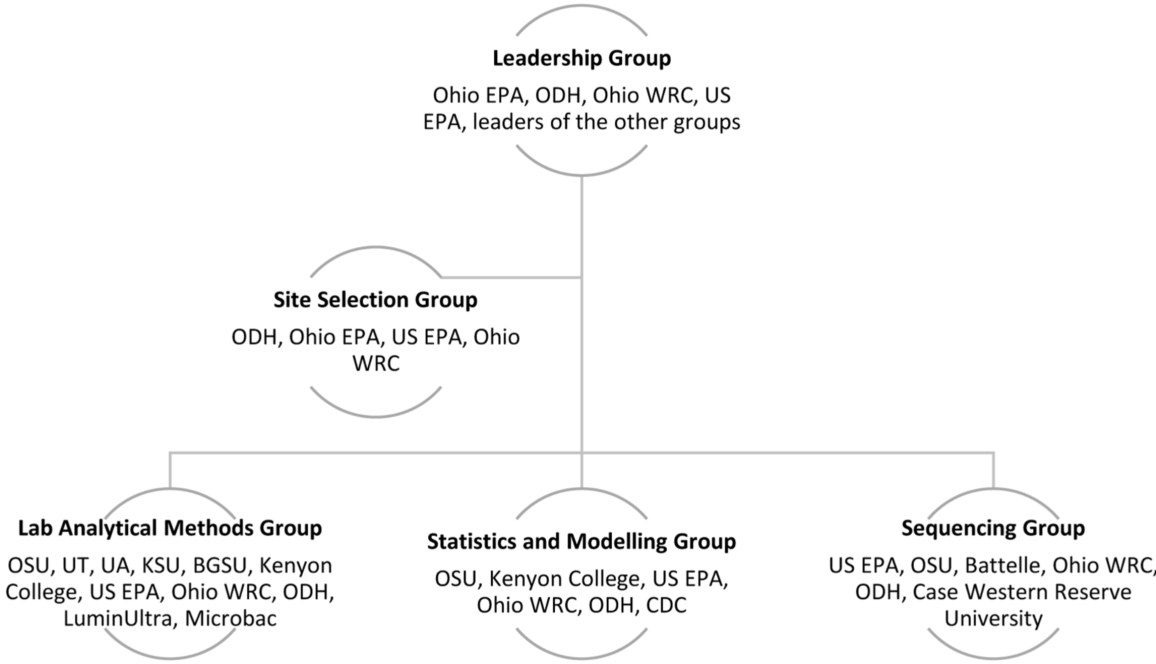

Figure 1.

The structure of the Ohio Coronavirus Wastewater Monitoring Network. Participating organizations included federal and state agencies (Ohio EPA, ODH, US EPA, Ohio WRC, and Centers for Disease Control and Prevention [CDC]), academic institutions in Ohio (OSU, The University of Toledo [UT], The University of Akron [UA], Kent State University [KSU], Bowling Green State University [BGSU], Kenyon College, and Case Western Reserve University), a non-profit research institute (Battelle Memorial Institute) and commercial laboratories (LuminUltra and Microbac).

Figure 2.

Wastewater treatment facility locations participating in the Ohio Coronavirus Wastewater Monitoring Network as of May 2022. The lines indicate the county boundaries with their names in the State of Ohio. The gray circles indicate the relative size of the population served per site. The black colored pins are locations recruited between July – October 2020, the white pins indicate locations recruited after October 2020.

Table 1.

Advantages and disadvantages of distributed network of research primary academic laboratories versus one centralized certified laboratory for sample analyses.

Advantages	• Easier to deploy quickly • Local resources are leveraged • Close to monitoring locations – easier sample delivery logistics • Relationship between lab and stakeholders often established • Resiliency in terms of supply shortages
Disadvantages	• Reliance on less experienced temporary workforce • Reliance on manual vs automated processes • Turnaround time capabilities dependent on the individual lab workflows • Results from different laboratories not comparable in the absence of standard method • Quality assurance and quality control variable among labs