SARS-COV-2 MONITORING IN WASTEWATER, A YEAR OF TESTING

Cristina Novo Pérez

Soon after the coronavirus swept across the world and millions of people went into lockdown in early 2020, we started learning more about wastewater-based epidemiology and its potential use to monitor COVID-19.

Wastewater-based epidemiology (WBE) is a promising field that detects pharmaceuticals, illicit drugs or pathogens in raw wastewater to provide population-level information. In this case, the idea was to monitor wastewater to detect the presence of people infected with SARS-CoV-2 in the communities under study.

Initially, sewage surveillance was heralded as a tool to detect the presence of the virus, as a complement to individual testing. The rate of increase/decrease in the concentration of coronavirus particles in wastewater over time can show trends, and that concentration could potentially be used to estimate the level of infection in a given area. Other emerging use cases are the possibility of identifying SARS-CoV-2 strains through sequencing work and thus track viral evolution, and

helping to track the effects of vaccination in a non-invasive manner.

Clearly, WBE holds a lot of promise. Research efforts are underway across the globe. As we enter the second year of the pandemic, to what extent is it being used to support public health responses? The Netherlands was the first country to implement a national wastewater monitoring programme for SARS-CoV-2, and many other countries have started or are planning to implement national-level monitoring. Besides national programmes, there are multiple instances of sewage surveillance at regional and local scales. The University of California Merced has set up an online compendium of wastewater monitoring efforts ongoing across the globe, the COVIDPoops19 Dashboard.

The Netherlands leads sewage surveillance efforts. The Ministry of Health, Welfare and Sport hosts a public dashboard with up-to-date coronavirus information, which includes weekly data on viral particles in wastewater per 100,000 inhabitants measured at about 300 locations. The data, provided by the National Institute for Public Health and the Environment, RIVM, is also corrected for the volume of water flowing into wastewater treatment plants at the time of sampling.

Also in Europe, surveillance programmes are in place in England, Scotland and Wales. The programme in England began sampling in 44 wastewater treatment works in July 2020 and expansion is planned to cover 80% of the population via monitoring at treatment works. Sampling by Scottish Water started in May, and the Scottish wastewater surveillance network includes now 28 sites covering 50% of the population, with data available through a public dashboard. Monitoring began in Wales in March with pilot studies that formed the basis of their surveillance program, which now encompasses 20 sites and 70% of the population.

Elsewhere, in Australia, testing is underway across the country, with different stages of development in different states. The ColoSSoS (Collaboration on Sewage Surveillance of SARS-CoV-2) project is an accelerated research programme supporting nationwide environmental surveillance for SARS-CoV-2. The goal is to provide data to COVID-control taskforces at a state level and share the data nationally.

Surveillance programmes intending to cover a broad area usually sample the influent to wastewater treatment plants, providing information about the area served

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by sewer pipelines collecting wastewater that is delivered to that single endpoint – the sewershed or wastewater catchment. Sewage sampling can also be done at different points within the sewershed – a major sewer pipeline or infrastructures such as pumping stations or holding tanks – and immediately at the sewer exit from an individual building or location. The latter, referred to as near-source tracking (NST), is used to monitor discrete populations, such as schools, university dorms, factories, prisons, elderly care homes, etc. Used in combination with targeted clinical testing, NST has clear potential to stop outbreaks and is being used in several countries.

Also along that line of monitoring a defined population, after detecting SARS-CoV-2 RNA in aircraft and cruise ship wastewater, researchers from CSIRO – Australia's national science agency – have suggested wastewater surveillance applied to transportation-based sanitation systems can provide additional data to manage viral transmission and may help these industries return to full operation sooner.

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What is the future outlook in terms of WBE's contribution to disease prevention and control? Many challenges remain, not only concerning wastewater sampling and analysis, but also data interpretation and communication. Studies show a correlation between SARS-CoV-2 RNA concentration (gene copies/litre) and positive COVID-19 cases in the community. However, estimating the level of infection in a community is one of the outstanding challenges of data interpretation. The normalisation of the results for the number of inhabitants and the dilution of wastewater (by rainwater or industrial discharges) provides a better indication of the level of infection in the population. One approach to estimate populations and account for wastewater dilutions is to use biomarkers (substances such as cholesterol, human genetic fragments or other microorganisms). Yet other sources of uncertainty fall within the realm of medical research, such as quantifying viral shedding rates and their dynamics over the course of the infection in symptomatic and asymptomatic individuals. Whereas estimates of the prevalence of coronavirus infection will require further research and integration of wastewater data with clinical testing data, the trend analysis of viral concentrations in wastewater already provides valuable information.

Nevertheless, some knowledge gaps identified early on are starting to be clearer. For instance, there is no evidence that water is a transmission route for the novel coronavirus. As predicted early in the pandemic, research to date has shown the virus is very fragile in sewage, and thus the risk of wastewater being a vector for transmission, even if untreated, is negligible. A study done in Italy on the presence and infectivity of SARS-CoV-2 virus in wastewater and receiving rivers detected the virus in some cases, which the authors attributed to non-treated or inefficiently treated discharges, or to combined sewer overflows; however, infectivity was null.

Research in this field is rapidly evolving, refining approaches and moving on to new priorities, such as the automation of analysis and the possibility of having data in real-time. At the same time, it has become clear that to realise the predictive potential of WBE so it can contribute to more proactive pandemic preparedness, communication between researchers, water utilities and public health decision-makers needs to improve. Buy-in by all parties involved is essential, particularly as successive waves of the pandemic are showing it is far from over. Next, we feature three interviews (you can read the full version in the Smart Water Magazine website) with experts in this field.

INTERVIEW

Dr Christian Daughton,
Formerly with the US EPA National Exposure Research Laboratory

"WBE stands out as the single most important missing tool in the armamentarium to control COVID-19, escape variants, and future pandemics”

Dr Christian Daughton, now retired, started to work for the U.S. Environmental Protection Agency as a research scientist in 1991. He has witnessed the evolution of wastewater-based epidemiology (WBE) research and application for different uses over some 20 years. About one year ago, interest shifted to improving its use for infectious disease monitoring, namely COVID-19. We interviewed him to hear his perspective on the potential of this not-so-new tool.

Currently, WBE is used to detect the presence of infections at the community level, and estimate relative change. Worth noting is that “to spot trends, sewage samples need to be analysed over a period of time sufficient to establish the background variability and signal noise”. It can useful “as a fast way in which to perform, en masse, population-wide random diagnostic testing at much lower cost”, says Dr Daughton.

WBE has not reached its full capability to monitor infectious diseases, estimating the actual number of cases in the community. As Dr Daughton explains "WBE cannot reach its full potential until an approach is developed to properly 'calibrate' WBE. Other measures will be required to benchmark WBE data to the actual number of COVID-19 cases." On the other hand, he highlights that "The biggest impediment to expanding the development of useful applications for WBE is the lack of government involvement". He calls for federal (and state) support and leadership in the United States, essential for making WBE a widespread, widely accepted tool in fighting pandemics.

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Asked about future research priorities, he thinks that "The future of WBE for infectious disease monitoring, therefore, needs to also focus on measures of the disease that target biomarkers produced by infected individuals as a result of the multifaceted disease itself." In this regard, markers of systemic or gut inflammation might have more predictive ability of numbers of infected cases and disease severity than viral loads in sewage, Dr Daughton explains. Also requiring further research, he points to "the need for real-time remote sensors capable of operating in sewage."

He is a strong supporter of wastewater surveillance as a tool for disease prevention and control: "WBE stands out as the single most important missing tool in the armamentarium to control COVID-19, escape variants (preventing the development of herd immunity via vaccination), and future pandemics." Looking ahead, he also points to its usefulness for "identifying populations having difficulty with accessing healthcare (because of racial or economic disparities). WBE could indefinitely continue to be necessary once COVID-19 becomes endemic because of recurrent epidemic cycles and promotion of new variants."

INTERVIEW

Professor Gertjan Medema,
Principal Microbiologist at KWR

"Environmental surveillance for SARS-CoV-2 has grown enormously, and is currently used in 50 countries and more than 1,000 cities"

Dr Medema, Principal Microbiologist at the KWR Water Research Institute in the Netherlands, pioneered early research on wastewater monitoring for SARS-CoV-2, using PCR methods to detect viral genetic material in sewage. SWM interviewed him in March of 2020 to learn about KWR's research in this field and what it could entail for the water sector. We contacted him again in 2021 to get an update on the status of environmental surveillance for SARS-CoV-2.

He explains that environmental surveillance for the novel coronavirus has grown enormously, and is currently used in about 50 countries and more than 1,000 cities worldwide. "It is increasingly being adopted by health authorities as a complementary source of information about COVID-19 circulation in cities, with several added values", he explains. Firstly, it is objective, because everyone goes to the toilet, whereas not everyone is tested. It is also fast, since virus shedding is high at the time onset of disease, and within a day it can be detected in wastewater. He also stresses its efficiency as a screening tool: "one wastewater sample (when sampled appropriately) can reflect from one thousand to one hundred thousand people. This is of particular importance when the prevalence of COVID-19 drops again to very low levels", as it occurs already in Australia.

Concerning challenges, Dr Medema notes that "the main challenge for sewage surveillance is that the water sector is not accustomed to providing data for the health sector and the health sector is not used to consider environmental surveillance when looking at public health"; nevertheless, more health agencies are getting involved.

As the pandemic evolves, more information is needed about the spread of new variants, and researchers are "focusing on

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tools to discriminate the new from the 'old' variants, using Next Generation Sequencing to look at all the virus variants in the virus mixture in sewage, and/or quantitative or digital droplet PCR assays with primers and probes to detect the variants/mutations/deletions in wastewater specifically". Wastewater surveillance will also show the effects of lockdowns and vaccination. "In this coming year we hope to see the disappearance of SARS-CoV-2 RNA from the wastewaters", says Dr Medema, as more and more countries move forward to the situation where Australia is now. Once disease prevalence is very low, "sewage surveillance becomes even more valuable to rapidly pick up if, where and when the virus resurges".

In the future, he thinks that the use of wastewater as a source of information will increase thanks to the pandemic, as a "mirror of society", for pandemics but also to track local outbreaks of disease, as well as to detect the use of pharmaceuticals and illicit drugs, etc.

INTERVIEW

Dr Zhugen Yang,
Lecturer in Sensor Technology at Cranfield Water Science Institute

"It is necessary to further understand the environmental dynamics, persistence and spread of SARS-CoV-2 to promote environmental surveillance"

In April of 2020 SWM contacted Dr Zhugen Yang, Head of the sensors laboratory at Cranfield Water Science Institute, to hear about his research towards the development of simple, low-cost paper-based devices for the detection of SARS-CoV-2 in sewage. We followed up with him again this year to find out about the state of affairs in sewage surveillance.

Dr Yang is positive about the role of environmental surveillance in the current pandemic, pointing to the fact that studies show a good correlation between viral RNA concentrations in wastewater and clinical cases of COVID-19. “Local and national monitoring programmes have been funded, for example on university campuses, in schools and national monitoring programmes in the UK, the Netherlands and Australia,” he reports.

There are, however, pending challenges concerning sample collection and treatment, analysis and data interpretation: “virus particles are usually unevenly distributed in the environment, so the differences between samples are usually large”. The gold-standard method, reverse transcription quantitative polymerise chain reaction (rt-qPCR), requires sample concentration, for which there is no standard method. Moreover, “the presence of certain chemical substances and biosolids can inhibit the PCR process, thereby differentially inhibiting the recovery efficiency of viral RNA and gene amplification”. Variables such as temperature, microbial activity and chemical substances can affect the rate of RNA degradation. “It is necessary to further understand the environmental dynamics, persistence and spread of SARS-CoV-2 to promote environmental surveillance for SARS-CoV-2”, Dr Yang concludes.

Future research can improve analytical methods to save cost and time: “low-cost and rapid sewage sensors (e.g., paper-based sensors) may play an increasing role”. This becomes even more important in resource-limited regions, without advanced laboratories. In addition, Dr Yang thinks it is necessary to study the shedding rate and duration of SARS-CoV-2, the relationship between the SARS-CoV-2 genetic signal (number of gene copies) and the prevalence of infection, and the impact of the environmental matrix on the virus.

Finally, he restates the benefit of wastewater monitoring as an early warning tool: “Studies have shown that an increase in SARS-CoV-2 RNA can be detected in environmental samples a few days before the clinical detection”. The same approach can be used for other infectious diseases, “by detecting the genetic biomarkers of disease for early warning”. Dr Yang notes WBE can be an effective and economic tool to monitor infections at the community level, “particularly in resource-limited regions, where insufficient medical resources will result in low clinical testing capabilities.”

Local and national monitoring programmes have been funded, on university campuses, in schools and in the UK, the Netherlands and Australia