Pathogens in the pipeline: How wastewater holds clues to the next pandemic

Melissa Suran:

This is Science Changing Life, and I'm your host, Melissa Suran. Think about the last time you washed your hands, took a shower or even flushed the toilet. You probably didn't give it much thought, but right now in wastewater plants across the world, researchers are analyzing what goes down our drains—not for what you might expect, but for hints about the next pandemic. Project scientist Josh Levy and PhD student Praneeth Gangavarapu are two of those researchers. They're using wastewater to track viruses like SARS-CoV-2 and H5N1, looking for new mutations before those variants make headlines. And as it turns out, what's in our sewers can tell us a lot—not just about the viruses circulating in our communities, but also about the future of public health. Josh and Praneeth, thank you both for being here today.

Josh Levy:

Yeah, great to be here.

Praneeth Gangavarapu:

Pleasure to be here.

Melissa Suran:

First of all, what is wastewater genomic surveillance, and how is it an effective way to track H5N1? I recall that method was also used to collect data on SARS-CoV-2.

Josh Levy:

Well, we got started with wastewater genomic surveillance here in San Diego as part of the pandemic response, working with a lot of our collaborators here in the area. So, this is especially the Knight Lab at UCSD, who were collecting wastewater samples. At first, they were testing those just to figure out how much of the virus was actually in the wastewater. And the next step was to get into the wastewater genomic surveillance side. And there wasn't this existing method for analyzing the sequencing data, so we put together an approach that allows us to infer the fractions of the different lineages that are spreading in the community and track those over time. So, this sort of allows us to not just dig into the information with respect to how much virus there is, but the specific variants and lineages that are spreading in the community, and their associated implications for public health.

Praneeth Gangavarapu:

The one great thing about this in tracking H5N1 is that we want to keep an eye out for possible mutations that may increase affinity towards humans. So, with wastewater surveillance, we're able to keep an eye out for these mutations. And then, since we're able to do that, we can

immediately isolate strains that may be more infective towards humans and then take appropriate public health actions to isolate them.

Melissa Suran:

So, why is wastewater surveillance so important?

Josh Levy:

Wastewater surveillance allows us to really expand the reach of our ability to monitor for infectious diseases in the community. Traditionally, this meant that we had to swab noses or take blood samples in order to track the spread of individual infections across the community. Wastewater allows us to monitor the entire community—up to millions of individuals from a single sample. And that allows us to direct public health responses really cost-efficiently, really quickly, in a way that is not possible through a lot of clinical surveillance efforts. This isn't always possible, but as a supplement to clinical surveillance, it's really effective to expanding the scale at which we can operate and the number of pathogens that we can track.

Melissa Suran:

Could you also walk me through how wastewater surveillance is typically conducted?

Josh Levy:

There's a lot of different forms for wastewater surveillance. It looks one way here in San Diego, where we're going to be collecting samples directly from a wastewater treatment plant. And that's pulling in waste from homes from across the city. We collect from three different sites here in San Diego, the largest of which covers about 2.3 million inhabitants. So, you have a single sample that's representative of that whole group. And then we're able to bring that sample into the lab, extract the specific virus components, and then actually quantify the amount of the virus that's in that sample. So, that allows us to get a sense of these measures of prevalence and incidence to understand exactly how much of the virus is in the community. And this is a paired approach when thinking about clinical surveillance, which is conventionally done with individual cases that are counted, usually following people testing. Of course, that's not always available. It's this supplementary component that allows us to have this redundancy in our public health surveillance.

Praneeth Gangavarapu:

What's also impressive about the wastewater surveillance is that in clinical surveillance, we have to do millions of swabs. And there's a certain amount of delay before data actually reaches the CDC and we aggregate that data. With wastewater surveillance, we're able to just take one sample from a wastewater plant, and we're able to immediately figure out what variants are spreading within that specific region. It's a more cost-effective method, and it's subject to lesser bias than clinical surveillance. But you do have to keep in mind that it goes hand-in-hand with clinical; they kind of are redundant data.

Josh Levy:

And one really important point here is cost-effectiveness. We're very privileged here in San Diego and in the US: We have the ability to really scale up our public health surveillance, especially in response to outbreaks like we saw during the pandemic. A lot of testing and clinical sequencing based on nasal swabs was done. That's not possible everywhere in the world. And a lot of the work that our lab does is in contexts where there's lower resources, and we have to

think a little bit more outside of the box, as to how do we actually monitor pathogens effectively in communities. So, we can, as I mentioned before, use wastewater as collected at wastewater treatment plants. But if we're creative about how we go about this, we can leverage things like topography, the flow of rivers, and where water pools, and where waste might be pooling as a result of environmental runoff, to actually get into the same level of insights.

So, we're able to do that—pretty much the same as we can do in San Diego—in a wide range of contexts by leveraging this. So, that works here in San Diego, of course, but we can do this in Nigeria; we can do this in Malawi. We've got longstanding collaborations there and across Sub-Saharan Africa, where we're doing very much the same.

Melissa Suran:

I'm glad that you brought up wastewater surveillance in Africa, since last year, you and your colleagues traveled to Nigeria to implement wastewater surveillance tools and procedures to detect viral pathogens. That being said, how do you globally track a virus like H5N1 to get international data trends?

Josh Levy:

We didn't quite focus on H5N1. This is more about establishing capabilities in some of our partner countries to perform wastewater surveillance, whether that's using wastewater treatment plants or using environmental collections. And whether that's for SARS-CoV-2, or for mpox or any of a number of different pathogens, having that capability is huge for establishing that ability to then transfer from COVID, et cetera, to other pathogens like avian influenza. And avian influenza is one that, once you have these capabilities set up, you're able to pretty quickly look in other key locations. That might be runoff from markets where you're going to see a lot of avian influenza, potentially higher risk in those areas.

But having people trained and able to do this sort of research and surveillance is a huge step towards building that underlying capacity. We are fortunate here at Scripps to have a lot of international collaborators that are working at the state-of-the-art surveillance approaches and methodological approaches. And we're able to bring that together and form best communities of practice, where we can establish what works and rapidly disseminate that information across all of our collaborators to be able to deploy these methods in the field immediately.

Praneeth Gangavarapu:

One such organization is PHA4GE [Public Health Alliance for Genomic Epidemiology] that really helps. So, in PHA4GE, we have a coalition of experts who come together and really help us make a standard framework, which is easily deployable, and people can follow and share data easily. Thanks to this, we can actually take a step forward towards pandemic preparedness because all these methods are accessible, and they're open and they can be reproducible. So, once they're deployed in various locations of the world, we're able to really track the evolution of various viruses, and then we're able to really prepare for potential pandemics. And once we immediately notice a strain that has pandemic potential, we nip it in the bud.

Melissa Suran:

How do your methods complement clinical surveillance approaches?

Praneeth Gangavarapu:

We do get clinical surveillance data of a number of cases, deaths from hospitals and other testing facilities. But they tend to have some amount of bias towards it—as in, people may or

may not go to the doctor, or some cases may not be reported. So, they are subject to some certain amount of bias. And even the samples and data we get, not all cases are sequenced, and we don't get all the genomic data for all the cases. That's where we see wastewater surveillance really come in—because wastewater surveillance is less subject to bias than clinical surveillance, so we're able to get a rough estimate of what the cases might be, the viral load, and we're also able to get it earlier. That gives us an advantage to really prepare for the next step. So, if you're seeing a slow increase in the viral load in the wastewater pipelines, then we immediately know, “Okay, the hospitals should prepare for an incoming wave of cases.” But again, I have to really emphasize that it complements clinical surveillance but does not replace clinical surveillance.

Melissa Suran:

It sounds like wastewater surveillance is well on its way to becoming even more efficient. But historically, why has it been so difficult to identify pathogens and their strains using this type of method?

Josh Levy:

There have been a lot of technological reasons why it was not really cost efficient or practical to do that. There's also the fact that it was thought that it was predominantly enteric viruses—things that are spread via the fecal-oral route—that were really detectable in wastewater. It's only fairly recently that we've been able to realize that actually quite a lot of things are actually shed from infected individuals and into wastewater, including respiratory pathogens like SARS-CoV-2. They will also be shed quite frequently into the waste and then are detectable.

But even so, they're at really low concentrations in comparison with a lot of other components of wastewater, so that kept people more focused on the individual cases. That's not to say that it wasn't used at all. Especially in the polio research world, there's been quite a lot of use of wastewater surveillance for many years. In just recent years, we've seen quite a few detections—predominantly of vaccine-strain polio. But still, this is prominently being detected through wastewater and then used in collaboration with clinical surveillance efforts to actually figure out which particular individuals might be involved and give them the appropriate care.

Melissa Suran:

Back to SARS-CoV-2, what scientific tools did you build during the COVID-19 pandemic that can now be used to track H5N1? For example, can you apply bioinformatics methods that you developed to other pathogens?

Josh Levy:

In response to this ongoing COVID-19 spread throughout San Diego, we developed this bioinformatic package that allowed us to do this inference of lineage prevalence. And we now call that Freya, which is a fun name that reflects our boss—Kristian Andersen's—Danish heritage. Freya ended up being widely used, and this is something that we made publicly available and allowed the community to use. And then unexpectedly, it really took off. And Freya sort of built on this very generalizable core method that has allowed us to leverage knowledge of lots of other different pathogens, to perform the same sort of lineage-type inference approach from wastewater collections that include other pathogens. So, that includes mpox, that includes seasonal flus, other respiratory pathogens like RSV, and most recently, H5N1. This is not just in wastewater. We can also do this in milk, which has also been a big point—especially now that we're dealing with a cattle outbreak, and the virus is detectable in milk. But yeah, that's one of the more recent applications we've been getting into.

Melissa Suran:

And H5N1 is mostly in unpasteurized milk?

Josh Levy:

Correct, yes. If you're drinking pasteurized milk, there's no danger to you. Although there are virus nucleic acids, these are not infectious whatsoever. Pasteurization has long been established and has shown time and again, including for H5N1, that there is no infectious virus following pasteurization. So, there's no risk to the general public, but we are still able to actually detect those nucleic acids and sequence them directly from milk.

Praneeth Gangavarapu:

Along with Freya, we have another tool called outbreak.info that was also created during the COVID pandemic. So, the advantage with that is we wanted to disseminate information but also aggregate information. They had three main things during COVID-19 they wanted to achieve: That was basically track cases and deaths; they wanted to integrate research publications, datasets, et cetera, into one searchable source; and then they also wanted to track SARS-CoV-2 variant trends. So, the outbreak.info website, that helped track all of this. It's been a successful website because we've had over 5 million page views and over 200 million API sessions. Researchers and public health officials—or anyone, actually—can view the website or access the data directly and pull their data and do their own analysis. So, it's more open source and easily accessible information. So, this really helps push the fact that we're able to disseminate information easily and quickly.

Josh Levy:

And these public resources are really important for the research community, as well as the broader public to stay informed and be able to determine their own best approach in terms of their own behavior and how they want to proceed—especially for immunocompromised individuals with our bioinformatic tools; especially working with our collaborators in low-resource countries. And this is really key to make sure that they have access to the most up-to-date tools, regardless of what amount of funding is available to their institution. So, a huge part of what we do is making our tools and our data as publicly available as possible and accessible broadly.

Melissa Suran:

On the topic of disseminating this type of information, especially when it comes to variant prevalence and identifying notable mutations, how do you know which variants to monitor?

Praneeth Gangavarapu:

There have been a number of studies that really explore how a mutation affects the virus in terms of transmissibility or pathogenicity. Recently, one such thing was the deep mutational surveillance that has started. A funny analogy would be: Imagine if you take an ice cream, and then you try different toppings and different ice creams—you would know which ice cream you like better. But then in deep mutational surveillance, we would take the ice cream and then make every combination possible. And you have like a ranking or a score for it. Instead, for us, it's going to be a virus. So, we have the protein—so, ice cream's a protein—and instead of tasting the protein, we decide to evaluate its transmissibility.

Because we get these scores for individual mutations, we're able to tell which strain has an increased transmissibility that's currently spreading out in the wild. So, the USDA deposits data into the NCBI, which is like a database. And from that, we're able to pull mutations and be like,

“Okay, this strain seems to have an increased transmissibility. And we have to keep an eye out for this mutation because we're starting to observe this mutation a lot more.” In such methods, we're able to quickly identify strains with increased transmissibility, but also mutations that are increasing in prevalence.

Melissa Suran:

That was a fantastic analogy, by the way. So, what are the local rules and guidelines regarding wastewater surveillance? And how can cities do a better job of enforcing them—beyond San Diego?

Josh Levy:

Praneeth earlier mentioned a little bit about PHA4GE, this Public Health Alliance for Genomic Epidemiology that we're a part of. And through this organization, we're trying to help craft a lot of the ethical and legal standards for the community. We aren't in the policy world or anything like that—but trying to leverage existing public health legislation and guidelines in order to understand what we can and cannot do, and what is ethically going to lead to key increases in distributive justice or increases in better public health access, and monitoring for communities that might otherwise be largely neglected by existing systems.

So, these ethical considerations are very much part of some of our collaborations through PHA4GE. But to be perfectly honest, a lot of standards don't exist in this space. Wastewater is sort of a unique modality for surveillance, in that it doesn't necessarily have a clear identifiability issue. You don't necessarily have clinical cohorts where those individuals are clearly sampled. Instead, you're sampling from a broad community. This can change as you decrease the spatial scale at which you're operating. So, certainly, at UCSD, we're doing building-level surveillance, where all of a sudden, there are much more identifiability concerns. And actually, running a public health surveillance program at that scale requires a lot more engagement with getting confirmation from students and from university officials to make sure that that's okay, and pursuing this broader community health goal.

Melissa Suran:

In what other ways do you hope future research improves wastewater surveillance?

Josh Levy:

I think there's a lot of key components that could really expand what we can do with wastewater; really starting to integrate that more with a lot of our core phylodynamic theories. And this is using sequencing data, the timing associated with that, the locations those samples are collected, in order to get this rich, dynamic picture of how spread and evolution is happening across geographic and temporal scales. I think bringing wastewater in a more intimate way into that framework will really be very powerful for how we understand outbreaks. So, I think that is a huge route going forward.

The other—to piggyback a little bit off of what Praneeth was talking about before—is really bringing all of these components together. So, you've got epidemiological information from wastewater, but you also have it from clinical. And you have genomic information from nasal swabs, but you also have it from wastewater; and these are sort of telling different parts of the same story. And using that in a public health setting to really best capture what's happening in the community and where we might be missing ongoing spread, either in clinical or via wastewater—because wastewater doesn't necessarily always cover everyone—to really understand this holistic picture of pathogen spread and evolution in the community.

Melissa Suran:

And in your opinion, how is Scripps Research unique in the way it approaches wastewater surveillance of pathogens, including viruses like H5N1?

Josh Levy:

We have a very unique research environment here, where we have access to some of the top-notch researchers in the world of immunology, microbiology. And we're able to access top-of-the-line, state-of-the-art sequencing technologies and laboratory instruments that allow us to operate at scale and work with a variety of different people all of the time. That fosters an environment where we can really bounce ideas off of people and think about the next key steps—whether that means starting to think about, “Can we address community immune status?” Thinking about antibodies as observed through wastewater, or other aspects that might be more relevant to understanding future evolution of the virus.

So, thinking about deep learning-based approaches to understand possible novel directions of evolution and their associated implications for the fitness of the virus, which can have many different interpretations depending on where you are, sort of, in the stage. H5N1 has a lot of different meanings of fitness when thinking about that with respect to say, SARS-Cov-2, which is already a pathogen that's circulating—human-to-human transmission is widespread. H5N1 is not that way.

So, being able to leverage all these different perspectives that we have here at Scripps and tools that we have at hand, that allows us to start thinking about, “Okay, we've seen this mutation. Is it associated with mammalian adaptation or human adaptation?” Or, “Does it stabilize the HA region of the virus, such that it might be able to be transmitted via a respiratory route?” These are key questions that we can work with some of the other labs here at Scripps to investigate in the lab. And then in our lab, we're really doing a lot of large-scale collection of sequencing data to see what's actually happening in the real world and how that relates to what we're seeing in the lab.

Melissa Suran:

Before we wrap up, is there anything else that either of you would like to add? We've covered a lot today, but is there anything important that we haven't touched on?

Josh Levy:

With respect to H5N1 in particular, a lot of these samplings from reservoirs, like cattle—these sorts of analyses, which in many ways have been the bread and butter of our lab for many years—are the analyses we need to be doing right now. Wastewater is this larger community monitoring approach. We're not necessarily expecting to see much H5N1 in wastewater. When we do, it's sometimes a little difficult to actually interpret the significance of that because it could be coming from runoff from a bird, or it could be coming from cattle. Sequencing does give us some insight into that, but it doesn't necessarily mean that it's coming from a human case. So, we have to be careful that we use the methods that we have at hand. A lot of the time, that is individual clinical sequences or sequences from infected animals to understand that phenomenon and move things towards an appropriate public health response.

Just because we see it in wastewater doesn't necessarily mean, at least at this point, that we're dealing with human-to-human transmission. In fact, that's very unlikely. It's much more likely that we're seeing some milk that someone flushed down the drain, and at this moment is not necessarily something we need to worry about.

It's really these sorts of core laboratory analyses; understanding the significance of individual mutations, and then how they might impact things like ongoing vaccine development and deployment. We certainly don't want to scale up a vaccine if we know that perhaps one of the virus clades that we see circulating, a virus lineage within that may not be well neutralized by that particular vaccine.

Similarly, with antivirals. If we're looking at something like Tamiflu, which is commonly used as an antiviral for avian influenza—or many different flus, in fact—it can be potentially neutralized by associated mutations in the virus. We want to make sure that our genomic surveillance directly links into the public health side of things, and also a lot of the ongoing drug development efforts here at Scripps, to best advise those as best we can.

Praneeth Gangavarapu:

In addition to that, the mutational surveillance we are doing at the lab—along with a lot of other collaborators—thanks to that, we quickly spotted a mutation that was increasing in prevalence. And then that was actually reducing the effectiveness of a current vaccine candidate. And it's all because of these collaborative surveillance efforts that we can do this mutational surveillance and really keep an eye out on the current outbreak.

Melissa Suran:

Lastly, to close things out, what's one surprising fact about wastewater surveillance?

Josh Levy:

Wastewater surveillance is wildly flexible. We've talked a lot today about operating at this large scale of entire cities in a single sample, but we can use wastewater surveillance anywhere from the level of individual buildings to the whole City of San Diego—and this can be used in very targeted ways. So, if there's a nursing home where we're worried an outbreak could lead to significant public health issues, we can monitor specifically that one building to make sure that no outbreaks go undetected and lead to potential disease spread across the community.

That also means that we're able to use this in a whole variety of contexts. So, we can use this to monitor migratory birds as they move from place to place. We can also use this to monitor communities that might not have formal sewage infrastructure. So, by using some creative approaches, we're able to collect in slightly different ways and do the same sorts of analyses that we would for wastewater at the community scale.

Melissa Suran:

And that's the latest on wastewater surveillance. Thanks again to Josh and Praneeth for sharing their insights on its growing role in public health. Be sure to check out the show notes for more information on the cutting-edge work at Scripps Research and exclusive content from behind the scenes. If you like what you heard today, please subscribe or leave us a comment. Thanks for tuning in and catch you next time on Science Changing Life—where listeners come curious and leave informed.