BIO Integration

Voice series: Interview with Dr. Doris Di, University of Hawaii at Manoa; frontier in COVID-19 detection from wastewater treatment

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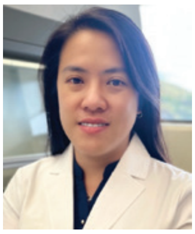
Foreword



Dr. Doris Yoong Wen Di has more than 12 years of experience in the study of environmental microbiology. She received her Bachelor's degree from University Malaysia Sabah in Environmental Science, and Master's and PhD degrees from Gwangju Institute of Science and Technology in Environmental Engineering. She is currently a postdoctoral researcher at the University of Hawaii at Manoa (UH Manoa). Her research interests include the microbial ecology of clinically pathogenic bacteria and viruses that are responsible for community diseases, and the spread of antibiotic-resistant bacteria causing antibiotic-resistant infections in the environment. She started her microbiology research career by studying the biodegradation of phenol by thermophilic bacteria. Shortly thereafter, her research focus changed to microbial-source tracking of fecal indicator bacteria in the environment, including *Escherichia coli* and Enterococci. She also studies the diversity of *Salmonella* spp., *Campylobacter* spp., *Klebsiella* spp., methicillin-resistant *Staphylococcus aureus*, bacteriophages, and many other species in environment samples. One of her most notable projects was a long-term study on *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* in coastal water, investigating their spatiotemporal-diversity changes, virulence factors, serotypes, and antimicrobial-resistance patterns. Interestingly, *V. cholerae* and *V. vulnificus* showed seasonal specificity and were observed in the environment in warm seasons, whereas *V. parahaemolyticus* was observed in all seasons throughout the year [1, 2]. She also found a "superbacterium" in urban river water—a carbapenem-resistant bacterium called *Klebsiella variicola*, which causes intra-hospital outbreaks of sepsis and fatal bloodstream infections. These bacteria carry New Delhi metallo-β-lactamase variant 9 (NDM-9), which confers resistance to β-lactams and a wide range of antibiotics including those used as last resort treatments [3]. This bacterium became a critical emerging issue after it was first identified in an environmental sample, and it received local attention and was the subject of interviews, features, and broadcasts on a Korean television program. Dr. Di has extensive microbial and molecular experience in the analysis of various types of environmental samples, including soil, fresh water, seawater, wastewater, bio-waste, and air, as well as samples influenced by natural events such as hurricanes, typhoons, floods, and seasonal changes. Over the years, she has received numerous awards and honors including the Outstanding Student and Strengthening Research Capacity Award, Top Student (2nd Prize) of the Global University Project, outstanding student presentation awards (oral and posters), Brain Korea 21 Research Fellowships, a POSCO Foundation Scholarship, and a Korean government scholarship. She has published 11 internationally recognized research articles and has four manuscripts currently in revision.

Dr. Di's most recent research focuses on the wastewater surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus strain causing the disease COVID-19. Since the start of the COVID-19 pandemic, she has worked on developing rapid and sensitive methods for detecting SARS-CoV-2 in municipal wastewater, to provide early detection of COVID-19 disease signals at the community level. She and her team worked with the City and County of Honolulu during a statewide lockdown at the beginning of the pandemic to investigate the trends in SARS-CoV-2 in samples from nine wastewater treatment plants, which process wastewater for approximately 1 million people on the island of Oahu. This surveillance report, published in *Water Research*, described tracking the daily dynamics of SARS-CoV-2 RNA in the wastewater from two large wastewater treatment plants in Honolulu during a rapidly expanding COVID-19 outbreak and a 4-week lockdown that resulted in a rapid decrease in daily new clinical COVID-19 cases [4]. The wastewater SARS-CoV-2 RNA concentrations exhibited significant inter-day fluctuations and an overall downward trend over the lockdown period, and the solid wastewater fraction contained the majority of the SARS-CoV-2 RNA mass. Dr. Di's team also worked with UH Manoa for early detection of the spread of COVID-19 in a student dormitory during a lockdown period. This project

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was aimed at detecting an early SARS-CoV-2 RNA signal in the wastewater from the dormitory, to enable rapid isolation of students before any apparent symptoms or positive tests for COVID-19, and prevent further viral spread. Currently, she is working on comparing rapid and efficient methods of SARS-CoV-2 recovery in wastewater and on developing a nested amplicon sequencing method to rapidly detect SARS-CoV-2 variants (Delta, Omicron, and others) in wastewater.

EE: Congratulations on your exciting episode of The Conversation, "Hawaii is ramping up wastewater surveillance for COVID-19," which was broadcast on Hawaii Public Radio. Could you please tell us a little bit of background on how this whole idea started?

DYW Di: The idea of using wastewater to monitor COVID-19 at the community level came from the use of wastewater to monitor microbial infectious diseases in human communities back in the 1950s, which originally focused on enteric bacterial pathogen outbreaks. More recently, more wastewater surveillance approaches for many potential applications have been explored, including poliovirus eradication, enteric disease outbreak detection, understanding the diversity of microbial pathogens in human communities, and antibiotic-resistance-gene distribution patterns. Moreover, there have been studies reporting the detection of SARS-CoV-2 viral RNA in fecal shedding from infected patients, and the detection of SARS-CoV-2 RNA in municipal wastewater was also reported in some countries at the beginning of the pandemic. At that time, the COVID-19 cases in the City and County of Honolulu were not severe, so we thought that SARS-CoV-2 wastewater surveillance would be a powerful public-health monitoring tool to track community COVID-19 disease burden.

EE: What was your major concern during the progress of this research? Did you face major stumbling blocks along the way? How did you overcome these problems?

DYW Di: There were a lot of struggles and difficulties along the way in this research, especially at the beginning. As you know, SARS-CoV-2 is a new emerging virus that is spreading rapidly in the community from airborne water droplets, person-to-person contact, or indirect contact with contaminated objects. Safety issues were the priority in initiating this research. Even though we knew that the rates of contagion of COVID-19 from wastewater samples were very low or near zero, we still had to be extremely cautious when handling and processing the samples. I had to go through a series of safety trainings (including respirator training, laboratory safety training, and so on); get medical clearance from physicians; be fully immunized against all possible exposure diseases, including influenza, hepatitis A and B, and Tdap (before vaccines for COVID-19 were introduced); and get permission and approval from the UH Manoa Institutional Biosafety Committee before I could start working. These took some time. When my team and I were developing the viral concentration and detection methods in wastewater, we also faced a lot of challenges. Although this virus is a strain of SARS coronavirus (severe acute respiratory syndrome coronavirus 1), the study of SARS coronavirus in wastewater was very limited, because this virus emerged and disappeared in a very short time (November 2002 to May 2004), and had a lower infection rate than SARS-CoV-2. The physical properties, survivability, partitioning behavior, and many other characteristics of SARS-CoV-2 in wastewater were not very well studied at that time. We had to find references and methods that were used to detect and characterize enveloped viruses (like human coronavirus and avian influenzas), because SARS-CoV-2 is an enveloped virus, and adapt viral concentration methods that were used to recover non-enveloped viruses (more common viruses present in wastewater). We utilized the molecular detection methods developed by the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO), but we had to optimize these assays and compare which assays were more reliable and applicable to SARS-CoV-2 in wastewater, because these assays were developed for clinical detection in human specimens. We also faced some problems in getting supplies, such as experimental reagents, chemical materials, laboratory supplies, and also personal protective equipment (including face masks, face shields, and gloves). Because that was a global lockdown period, the whole world was prioritizing these materials for hospitals. Deliveries were delayed as logistics supply chains were interrupted, and mostly every material needed had to be imported into Hawaii. I just did my best with everything I had. Every day had new challenges, and I just had to deal with it with patience. When we started processing wastewater samples massively and consistently, we also faced some manpower issues. First, not every person was authorized to handle wastewater samples. Second, we were processing wastewater samples, and at the same time enhancing and increasing the detection sensitivity of the methods we were developing. Plus, the research on SARS-CoV-2 is still developing and evolving rapidly every day through the efforts of scientists all over the world, and we have to get the latest information and adopt it in our research. It was like burning candles at both ends back then. While most of the people were working from home, we had to come to the laboratory every day—just like the essential workers in the hospitals and clinical facilities—which also increased our risk of getting COVID-19. It was important that we made sure we did not get COVID-19 at all times, or else the wastewater research would have been halted.

EE: Could you elaborate on why you are looking into wastewater specifically?

DYW Di: Typically, we know that wastewater is loaded with enteric pathogens (microorganisms such as bacteria, viruses, and parasites) that cause intestinal illness, and it has been used to monitor microbial infectious diseases in communities. Wastewater surveillance of SARS-CoV-2 can provide an early warning of COVID-19's spread in communities. Wastewater consists of many types of human bodily wastes that contain the SARS-CoV-2 virus, in particular feces and urine. Since municipal wastewater collects wastes containing all COVID-19 infections, including asymptomatic, mildly symptomatic, pre-symptomatic, and symptomatic ones, it has the potential to enable comprehensive surveillance of disease transmission in a community. Once *health departments are aware, communities can act quickly to prevent the spread of COVID-19. Public-health officials can determine if infections are increasing or decreasing in a sewer shed or in a community, too. Unlike any other types of COVID-19 monitoring, wastewater surveillance does not depend on people having access to healthcare, people seeking healthcare when sick, or the availability of COVID-19 testing. Especially right now, more and more people are utilizing home testing kits, so there will be a lack of data at the clinical testing facilities, but wastewater captures all the viral shedding into the wastewater, and provides robust and genuine real-time infection data. Clinical surveillance could also be slower because of the overwhelming number of patients and testing samples, limitations in testing equipment and manpower, or prolonged outbreak detection due to unclear reporting guidelines or communication issues.*

EE: Would you say that the approach that you have undertaken is the best measure to date? What would you have done differently if given a chance to revamp this project?

DYW Di: I would say that the approach that we developed is a comparable measure to other approaches developed by many others with the existing resources and equipments we have in our laboratory. During the time we started this project, we could utilize only the existing resources and equipments, facilities, and supplies we had in the laboratory, because we need to fight with time. The faster we have a method, the faster we can start wastewater surveillance in communities. Fortunately, we were able to develop a rapid, efficient, and cost-effective method for detecting SARS-CoV-2 in wastewater. There are a lot of methods, devices, and machines developed and introduced in the industry today for the same purpose, which is to detect SARS-CoV-2 in wastewater samples. However, you need to have funding and a budget to purchase the devices or machines, the supplies needed would cost money, and ordering and delivering these items would take time. The method that we developed uses reagents, chemicals, and supplies that are easily found in a microbiology laboratory. The turnaround time is short, and the data that we obtained are reliable. The cost is very low, too. The method is simple and easy to conduct. Of course, we are still trying to improve the detection sensitivity and limit of detection, because wastewater samples have a complex component and contain many unknown inhibitors that limit the detection sensitivity. If I had a chance to revamp, I would still choose an easy method that would provide accurate and reliable results with a short turnaround time. Plus, I would probably hire and train more people to improve the work efficiency and reduce the workload. We were not a big organization, so we basically did everything, from setting up autosamplers, collecting wastewater samples, processing wastewater samples, analytical measurement, data interpretation, report writing, and so on. All of this takes time, and what we were fighting with was time. We used to have two projects going on at the same time, so that was probably the thing that I would want to do differently if I had a chance.

EE: From your CV, we understand that you came from a microbiology background. When did you start venturing into wastewater treatment? Are there difficulties in the integration of these two fields?

DYW Di: Well, I had experience with wastewater research during my graduate study. One of the projects was wastewater surveillance of antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARG). We collected and processed the wastewater from the influent, which is the stage where the wastewater flows into a treatment plant from the communities, and identified the ARB and ARG. Surprisingly, we were able to capture a whole lot of ARB and ARG in the wastewater from the local community. I was also involved in a global sewage surveillance project with WHO that aimed to collect ARG distribution patterns in wastewater all over the world. Another project was to investigate the fate of ARG during wastewater treatment, specifically the inactivation efficiency of plasmid-encoded ARG in both extracellular and intracellular (within bacteria) forms during water treatment with chlorine, UV, and UV/H_2O_2 [5]. I have been doing research on microbiology in various kinds of environmental samples; wastewater is another environmental sample for me. I did not find it difficult. The things that I needed to know were the research purposes, hypothesis, targeting microbes, and methods before initiating the research. In fact, I think that wastewater is a very interesting and valuable environmental sample, which provides a lot of information, especially related to public health. With my experience in working with the pandemic virus, I really think that integration in various fields is important for resolving global issues like COVID-19, especially in wastewater research. I know that a single-discipline investigation is inadequate to respond to the global outbreak of COVID-19. Basically, it needs pathogen identification (epidemiology and virology), virus screening (omics and imaging/genetics), vaccine development (biomedicine and molecular biology), and diagnosis and therapeutics (clinical medicine and pharmacy) [6]. We need information, experts, and professionals from different fields (physicians, policymakers, researchers/scientists, health officers, and communities) to work together and try to find out more about this virus, to contain the spread of this virus, to develop detection methods for this virus, to develop vaccines for this virus, and so on. In the research field itself, I also encountered that having interdisciplinary integration really helps in speeding up research on SARS-CoV-2. For example, we needed information from the medical fields about the survival, spreading, and shedding rate of the SARS-CoV-2 in order to incorporate the information into our research designs and concerns. We also required actual COVID-19 infection rates in the local community in order to validate the data we obtained from wastewater. However, when we were testing the wastewater of the local community to quantify SARS-CoV-2 RNA related to the actual infection rates, we encountered a problem where the state refused to release nasal-swab testing data for the zip codes that feed into specific wastewater treatment plants, due to privacy concerns. This did obstruct our research from going deeper into finding out the actual infection rates in specific zip-code locations; we could only validate the data as a whole, covering a big area. In my opinion, I think that if we could have had the data, health officials could have targeted the community in the specific zip codes by referring to our *data obtained from wastewater, and started testing everyone and implementing COVID-19 containment procedures in that area, and prevented further spreading of COVID-19 in Hawaii.*

EE: Do you envision that your approach to detecting COVID-19 in wastewater could be easily used by larger areas or countries? What are other possibilities for this research in terms of the detection of other harmful viruses in our environment?

DYW Di: Honestly, I do think that our approach to detecting COVID-19 in wastewater is easy, rapid, time- and cost-effective, and easy to initiate in any microbiology laboratory. As long as you have enough manpower and supplies, wastewater surveillance can be initiated, even for larger areas or countries, especially developing countries. Probably our method is more user friendly. Briefly, we adopted the adsorption-precipitation method in viral-particle concentration from wastewater, RNA extraction, reverse transcription, and real-time PCR. The turnaround time is approximately 9–12 hours, depending on the number of wastewater samples. This turnaround time has been improved from our previously developed method, which has a turnaround time of 3 days. In fact, in the United States, the CDC has launched the National Wastewater Surveillance System, which coordinates with local health departments and builds the nation's capacity to track the presence of SARS-CoV-2 in wastewater samples collected across the country. It aims to transform independent local efforts into a robust and sustainable national surveillance system. Answering your last question, wastewater surveillance is able to detect all kinds of harmful bacteria or viruses in the communities or environment. The source of wastewater is mainly from the community, but some environmental factors could also contribute to the bacterial or viral input from the environment, such as rain or stormwater runoff. Many studies have reported the detection of genomic material from enteric pathogenic bacteria (Salmonella), enteric viruses (adenoviruses, polioviruses, enteroviruses, noroviruses, and rotaviruses), avian influenzas (H1N1), and many more. One of the studies in our laboratory has reported a strain of Salmonella enteric serovar Paratyphi B variant Java detected in the wastewater simultaneously with a reported clinical outbreak, and months later, it was detected again in the wastewater as the dominant pulsotype but with no corresponding clinical cases reported [7]. This showed that wastewater surveillance is able to detect non-clinical reported disease outbreaks in the community.

EE: What advice do you have for the young generation of scientists today?

DYW Di: For young generations of scientists, I would say: do what you like, and like what you do. If you chose the path of being a scientist, then you must have research passion in whichever fields you are in. Always be diligent, genuine, and follow the principles of research ethics. The process of research, or getting a degree (especially a Ph.D.) may be difficult and stressful, but it can also be fun, and you will feel contented when you overcome all the challenges. Looking back, all the challenges made me who I am today. I am not great, but I am stronger. I am also still learning along the way every day now. Pursue the life that you want without any regrets. Good luck!

EE: We would like to congratulate you on your current achievements. Do you have any advice for *BIO Integration* or to other editorial-board members (particularly our young batch of academic and youth editors)?

DYW Di: Thank you once again. My research interests and field are obviously different from most of the editorial-board members', but it would be my honor to get to know and learn some skills and knowledge from them. For BIO Integration, I would like to suggest including public health in the scope of this journal, because I think public health is deeply connected to medicine. In my research, I often refer to clinical case reports and existing diseases in clinical settings, because those data are strongly correlated to environmental or community disease findings.

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