ASIA PATHOGEN GENOMICS Initiative

DukeNUS Medical School | Centre for Outbreak Preparedness

Asia PGI Webinar Series #2

Wastewater Surveillance For Pandemic Prevention In Low-resource Settings

KEY INFORMATION AND FAQS

Asia PGI Webinar Series #2
Date: Monday, 26th August 2024

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Wastewater Surveillance For Pandemic Prevention In Low-resource Settings

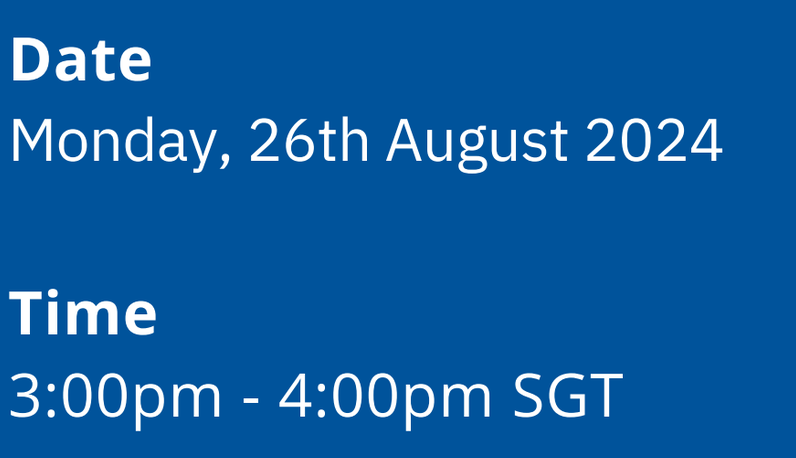
Sustainability of wastewater surveillance post-COVID-19 pandemic is at a critical juncture, especially in low-resource settings. *Swasti, The Health Catalyst* will be sharing their experience in establishing and sustaining a wastewater surveillance system in Bengaluru, India. Speakers will discuss:

- Status and challenges of implementing wastewater surveillance in Asia
- Stakeholder management in establishing multi-pathogen wastewater surveillance
- Wastewater sampling strategies optimization
- Building sustainable capacity for wastewater surveillance
- Translation of wastewater surveillance data for public health decision making

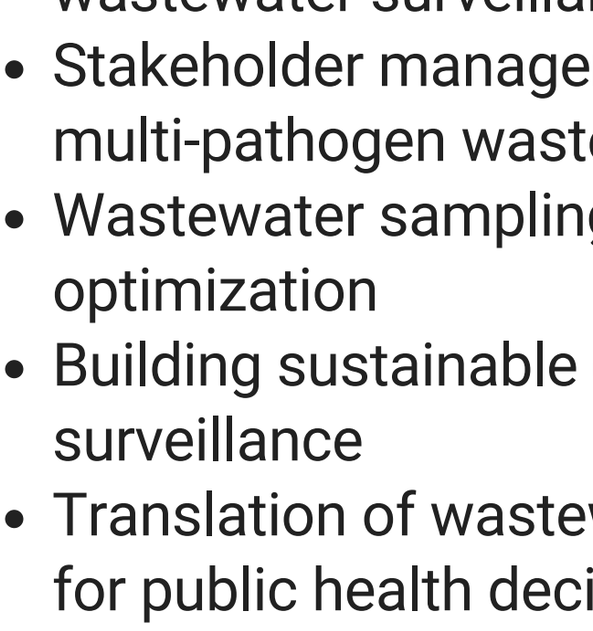
Date
Monday, 26th August 2024

Time
3:00pm - 4:00pm SGT

Watch the Webinar
https://www.youtube.com/watch?v=ZUcK9GrXwJU

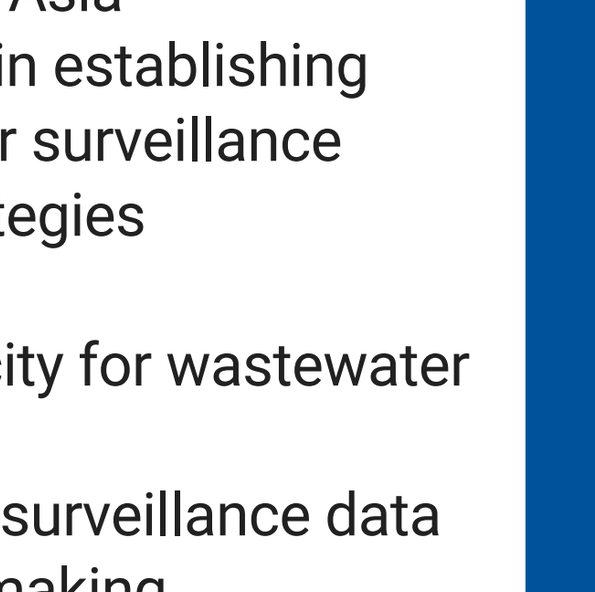


Moderator



Dr Pang Junxiong Vincent
Assistant Professor, Centre for Outbreak Preparedness, Duke-NUS Medical School

Speakers



Shirish Harshe
Senior Technical Specialist, Precision Health, Swasti

Sabhimanvi Dua
Project Manager & Communication Lead, Precision Health, Swasti

Key Information and FAQs

What is your experience with policy makers to use the platform especially as an early warning system and specifically what actions were taken?

- The policy makers at the state level were onboarded since the start of the project. We sensitized them about the concepts of WES and the outputs the surveillance initiative can generate. The policy makers at the state level constituted Karnataka Technical Advisory Committee (TAC) for COVID-19. They (TAC) recommended the state to approve the rollout of WES in the city of Bangalore.
- We could largely detect early warning signals only during the COVID times. As we have shown in our presentation in the webinar, we could detect cases of early warning signals in the last week of December 2021. Incidentally, the clinical cases also started to increase in the first week of January.
- The subsequent actions after reporting early warning signals were first validation of the data through clinical surveillance data, mandating masking in public places and intensifying clinical testing in all wards of the city. The private diagnostics centers and hospitals were also given additional responsibility of testing more samples.

What initiatives can be taken for sustainability to adopt this as a surveillance system in the govt system?

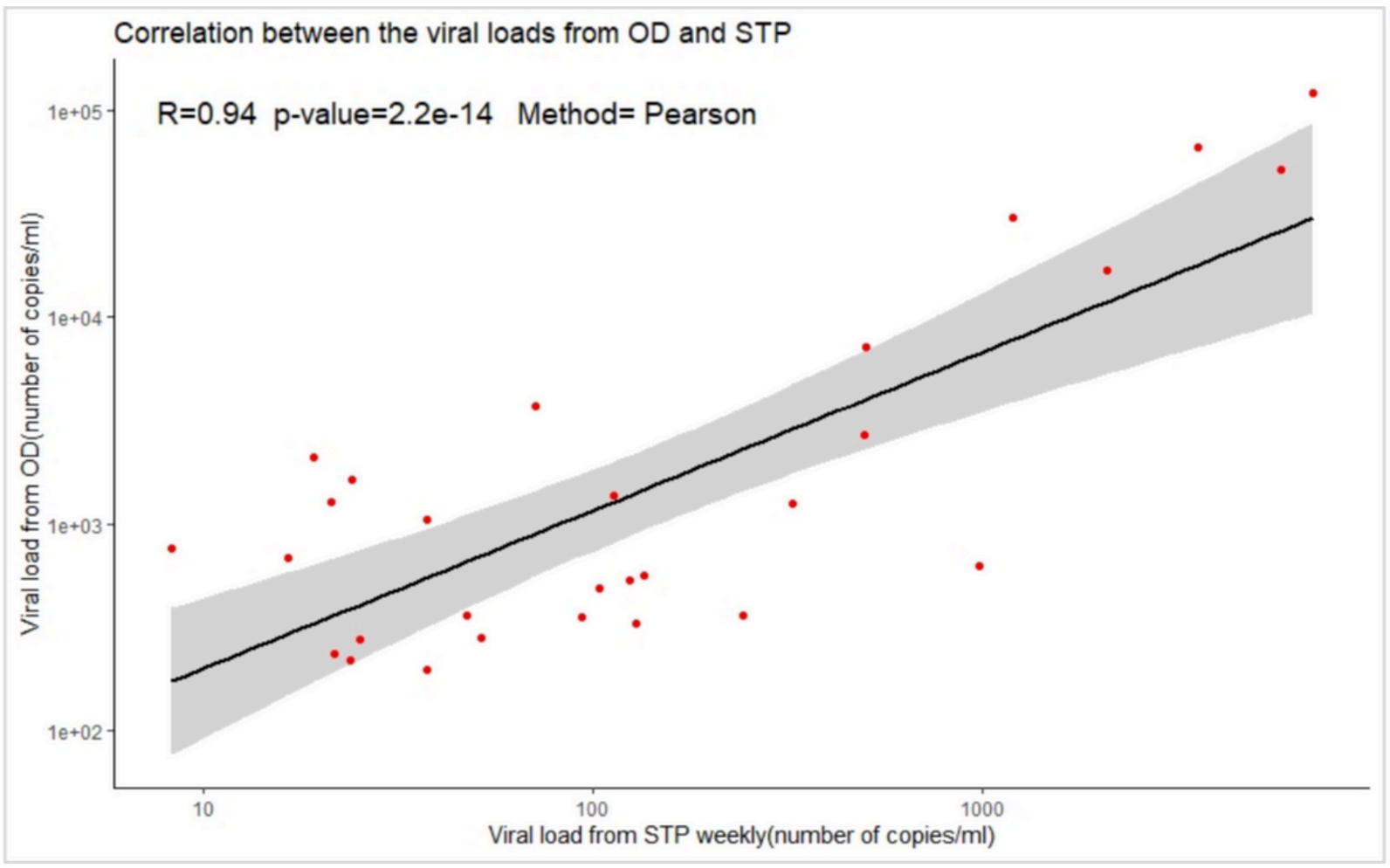
- A continuous WES in a city is a cost intensive process.
- However, with the help of other intelligence tools such as Event Based Surveillance (EBS), GIS and participatory surveillance, we can have a need-based surveillance as and when surveillance of a pathogen is required. This can decrease the cost of surveillance significantly.
- Institutionalization of WES in the government system is the most effective way for sustainability of WES. In our opinion, leveraging the current systems and infrastructure laid by the government is the best approach. The local government in India, which is accountable for supplying drinking water and treating wastewater, also has the responsibility of operating water testing laboratories to test drinking water. These samples are tested periodically to observe the change in composition. If the lab staff can get trained in the sampling and testing process in WES, with a one-time procurement of devices and a monthly rate of consumption of supplies (reagents and chemicals), this can be institutionalized and thus sustained for a long period.
- A state-level committee that observes the incidence of outbreaks at the district/state level, can look at WES data to detect early warning signals. They can also manage the initiative as a program, managing the funds allotted for WES.

Did you manage to connect the site wastewater surveillance data with clinical data from the sampling site?

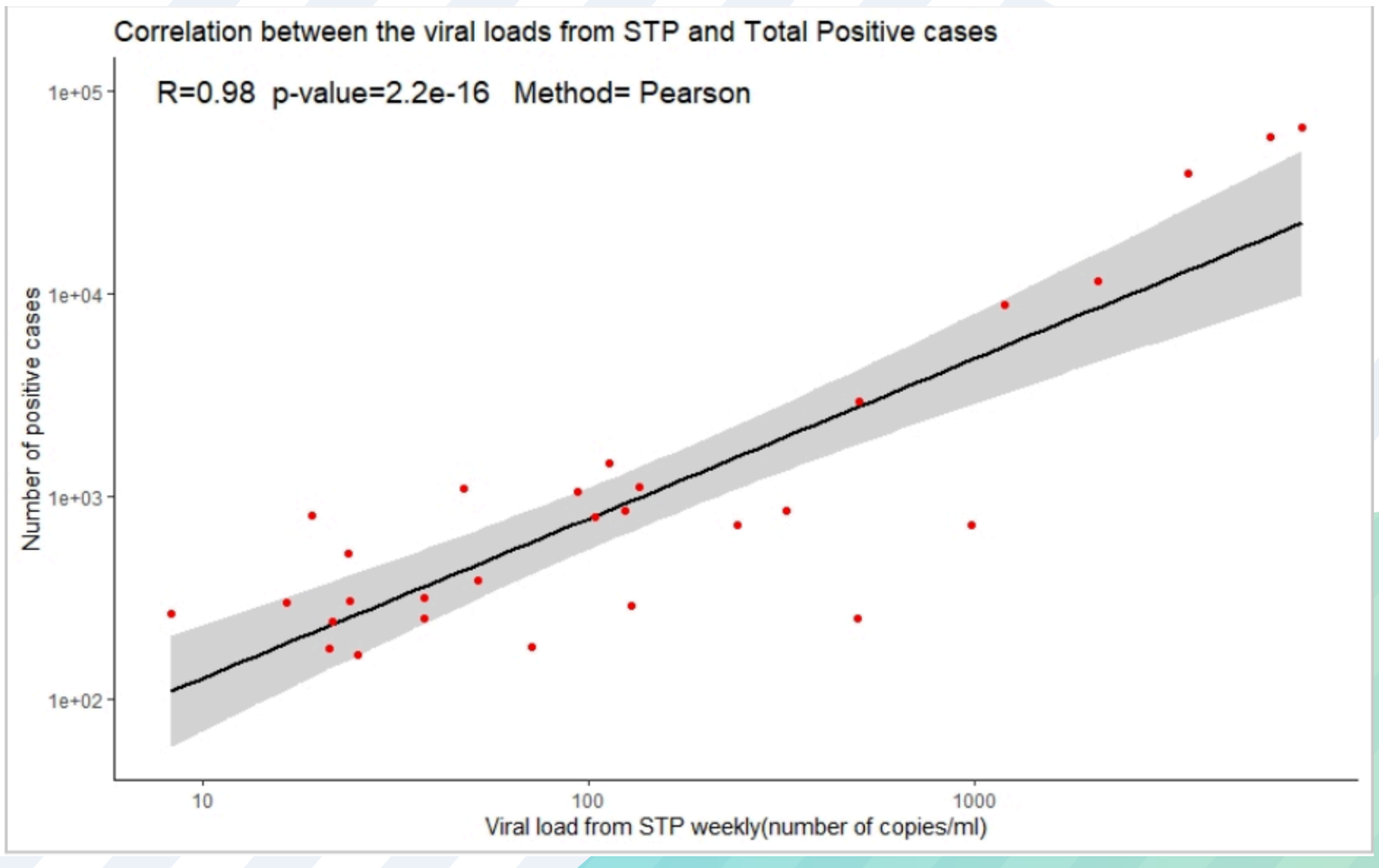
- Yes, we tested the concordance of WES data with respect to clinical cases and our study postulated a good correlation between the datasets. Below are the findings for our study:

Key Information and FAQs

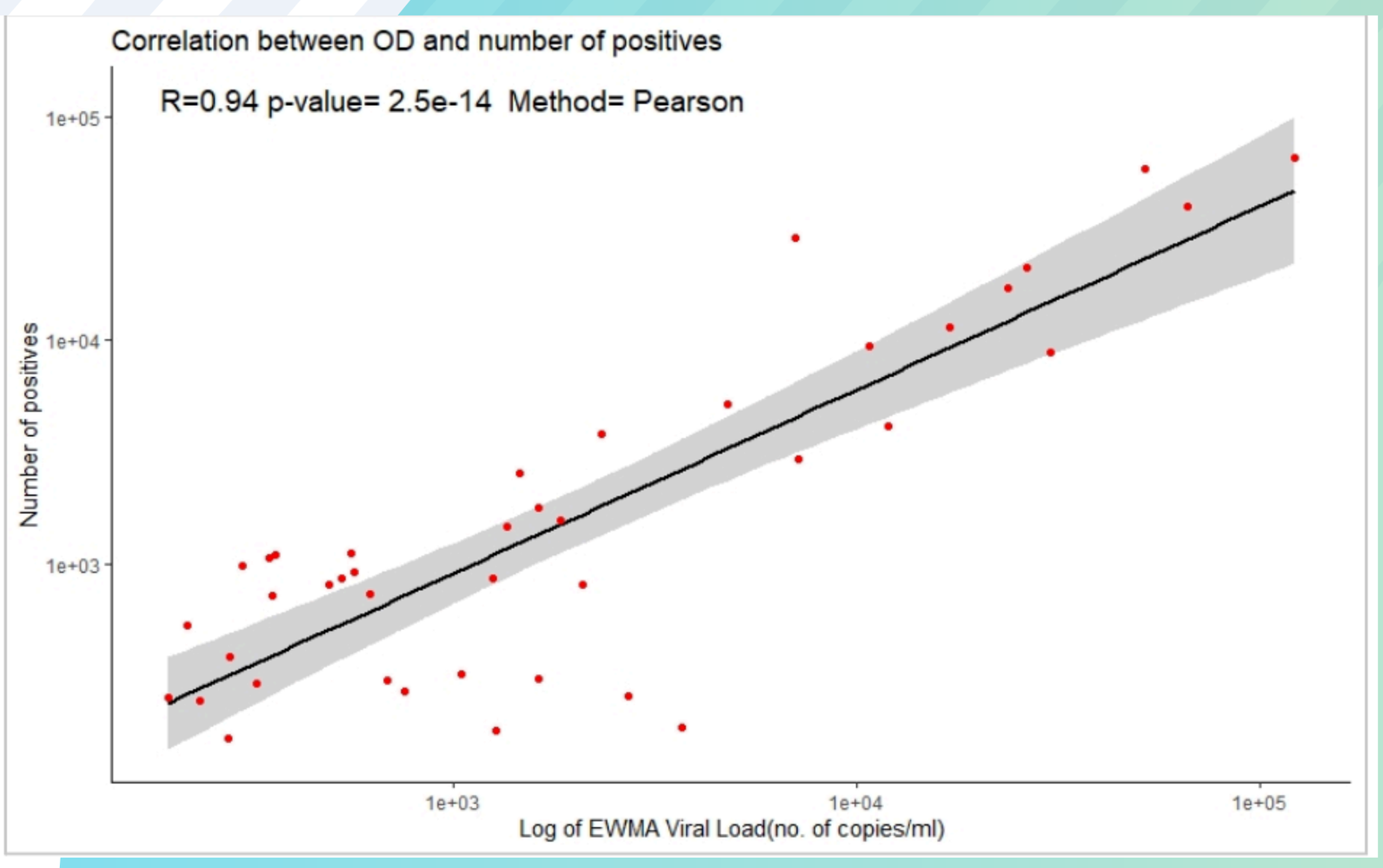
Positive samples from Open Drains v/s Positive samples from sewershed sites



Sewershed sites v/s positive clinical cases



Positive clinical cases v/s Open drains



Key Information and FAQs

Globally, there are various studies that show a high positive correlation between the WES data and clinical surveillance data. Some of the studies can be referred below:

1. **Metrics to relate COVID -19 wastewater data to clinical testing dynamics - Amy Xiao, Fuqing Wu, Mary Bushman**
2. **Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in The Netherlands - Gertjan Medema, Leo Heijnen, Goffe Elsinga**
3. **Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance**
4. **Systematic review of wastewater surveillance of antimicrobial resistance in human populations - K.K. Chau, L. Barker, E.P. Budgell**

What were the criteria for surveillance site selection?

- Selection of sites from the sewershed is straightforward. We collected samples from the inlet of each STP. The inlet of an STP offers the raw wastewater free from any treatment that can change the composition of the wastewater sample.
- In the open drain system, site selection was a concern. We adopted a simplistic approach to site selection, as at that time, the relevance was to detect positive samples around the city and not on detecting the source of the infection. The steps followed to select open drain sites were:

1. Identify wards/administrative blocks with most population
2. Identify spots/sites in which:
    - Within the proximity of high population density
    - Ensure that there is perennial flow of wastewater
    - Ensure that there is accessibility to draw wastewater samples throughout the year

We also took the help of a local wastewater drainage expert, whose inputs were vital in site selection. Eventually, we found that GIS can help in building sufficient rationale in site selection. GIS can help in site selection as it provides an understanding of the following:

1. Elevation mapping
2. Natural drainage network
3. Mapping of OD/STP sites
4. Mapping of Catchment area using elevation and natural drainage cut offs
5. Mapping of Residential area using Bangalore Development Authority Land use Analysis 2015.
6. Residential Neighbourhood Typologies (Three neighborhood types are identified based on their physical characteristics such as built density, tree cover, street characteristics)
7. Calculating total number of building (not only residential) in the catchment area (The number of buildings is based on Microsoft Building Footprint database downloaded in December 2022)
8. Population Estimates for 2022 (We use our own high resolution 2011 population data (generated from ward level census data) as a base for estimating the 2022 population totals.

Thus, satellite data on elevation, green cover, streets and natural drainage, demographic and socio-economic data , if available, with the support from data analytics and GIS experts can provide validated insights on prospective sites that can be selected for sampling.

Key Information and FAQs

How do variations in shedding rate, or decay of multiple pathogens in wastewater, and low pathogen abundance, influence the detection and quantification of these pathogens?

- All these factors certainly affect the outputs of WES.
- On shedding rate: A rising number of asymptomatic/unreported cases as the shedding rate increases leads to earlier wastewater signals than clinical surveillance. Furthermore, the higher the maximum number of unreported cases the more in advance the wastewater data from the medical data. More details can be found here: **Relating SARS-CoV-2 shedding rate in wastewater to daily positive tests data: A consistent model based approach.** Moreover, some people continue to shed pathogens in their feces after recovering from an infectious disease, and can still transmit the disease to others. Hence, while the clinical testing trend may drop sooner with the help of both preventive and curative measures, the wastewater surveillance trend may take longer time to drop.
- Decay of multiple pathogen: Pathogen decay in wastewater causes under-estimation of infections for WBE, especially at high temperatures. The decay rate of these pathogens can vary due to the seasonal fluctuation of wastewater temperature, dilution by rainwater inflow, groundwater infiltration and seawater intrusion in sewer systems. The decay also affects bacteria and viruses differently. The dilution is open drains to the addition of rainwater WBE sensitive to decay of bacteria but not viruses.
- The explanation of occurrence and abundance of viruses in wastewater can be referred in this study: **Viruses in wastewater: occurrence, abundance and detection methods**
- One should note that the low detection of pathogen(s) from wastewater does not necessarily correlate with low clinical cases in the community. This is due to variable rates of viral shedding from infected people. Hence, the variable shedding rates and decay kinetics may affect the detection of pathogens in wastewater. Among the common pathogens that cause infectious diseases, Norovirus, Coronoavirus and rotavirus are said to have a higher shedding rate, compared to the other pathogens. Hence, before targeting a pathogen for wastewater surveillance, all these factors need to be taken into account.

Did you use process control during the testing of pathogens from wastewater? If yes, please explain which one is commonly used?

- For sample inactivation:
- As per our internally developed SOP developed by our partner, the sample inactivation is conducted by putting the samples in a hot water bath at 60°C for 45 minutes. Hence, the incubation period in the water bath is 45 minutes.
- Research studies suggests:
- **Pasteurization on 60°C for 90 mins**, it is mentioned that “Upon initial receipt, samples were placed in the biosafety cabinet with UV for 20 min and then pasteurized in a 60°C water bath for 90 min to inactivate the virus. Previous studies showed that pasteurization could effectively inactivate the virus without compromising sample quality. Pasteurized samples were then used for viral precipitation, and the remaining samples were stored at 4°C.”
- **About heat inactivation**: “SARS-CoV-2 is relatively sensitive to heat inactivation under our laboratory conditions. These data can help laboratory workers to elaborate and improve their protocols for SARS-CoV-2 experiments, and reinforce our current knowledge on coronavirus survival.”

Key Information and FAQs

- **Recommendations on pasteurization:** The exposure to high temperatures can result in the denaturation or coagulation of vital cellular components, causing loss of viability. As per the study, pasteurization is recommended to maintain the quality of the sample.
- For Precipitation
- As per our internally developed SOP, to conduct sample precipitation, 50 ml of the sample is mixed thoroughly with PEG 6000 and NaCl and incubated at room temperature for 10 minutes.
- Research studies suggests:
- **Comparison of various precipitation methods**: The yields were significantly higher after Ethanol-NaCl precipitation. As per the study, “In comparison to Ethanol-NaCl precipitation, Isopropanol-Ammonium Acetate precipitation resulted in ~20–30%, Isopropanol-NaCl in ~20–50%, and PEG 6000-NaCl in 30–40% lower DNA yields. PEG 8000-NaCl and PEG 8000-Ethanol-NaCl performed the next best with only ~10% lower DNA yields.”
- **Evaluation of precipitation methods**: PEG-NaCl precipitation method was compared with ultracentrifugation, and skimmed milk flocculation. As per the study,
    a. PEG precipitation and PAC flocculation protocols were efficient for SARS-CoV-2 concentration and recovery from wastewaters.
    b. However, PAC flocculation showed a lower limit of detection (4.3 × 102 GC/mL) than PEG precipitation (4.3 × 103 GC/mL).
- For RNA extraction:
- In this initiative, RNA extraction is conducted through spin column based nucleic acid purification.
- As per our internally developed SOP, the pellets after centrifugation are pipetted out in microfuge tube and specific quantities of Proteinase K, Carrier RNA, as well as Ethanol is added in different phases for further centrifugation cycles. The NBB and NWB are also added for separate cycles of centrifugation.
- Research studies suggests:
- spin column-based nucleic acid purification using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and
- acid guanidinium thiocyanate–phenol–chloroform extraction using TRIzol reagent (Thermo Fisher Scientific, MA, USA).
- In this initiative, RNA extraction is conducted through spin column based nucleic acid purification, which is proven to be a better method by some **studies.**

Key Information and FAQs

Have you tried some other collection methods than Grab, likes of BMFS, more swab etc.? Additionally, for detection are you using un-targeted mNGS or any panel testing?

No. We have not tried any other sampling methods in wastewater sample collection given the low resource requirement as compared to composite sampling. This allowed us to implement frugal innovation at scale in the cities of operation. We have yet to explore other techniques of sampling in the Indian wastewater context. We have also not used mNGS or any panel testing and most of our partners who used NGS for testing wastewater samples do targeted sampling.

What other zoonotic diseases did you detect and were you able to correlate it with the clinical data of the disease?

The platform mainly tested SARS-CoV-2, Influenza A & B, H1N1, Hepatitis A & E, Mpox and AMR. Mainly SARS-CoV-2 and Influenza A & B and H1N1 were correlated with clinical data of the disease.

Can we actually do WES by taking samples from a potentially contaminated river flow in the urban population?

Yes we can. We actually did something similar in the lakes. The objective and scope would be different though:
https://swastihc.maps.arcgis.com/apps/dashboards/a19e312232c94821bd67b2bee315ef50

For more information, please contact our panelists:

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