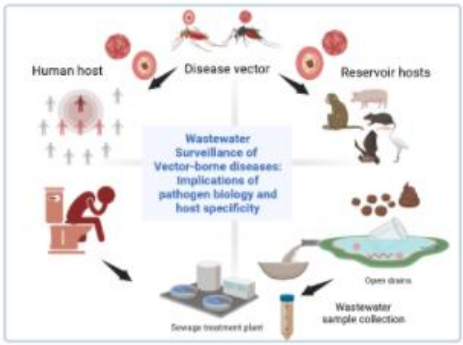
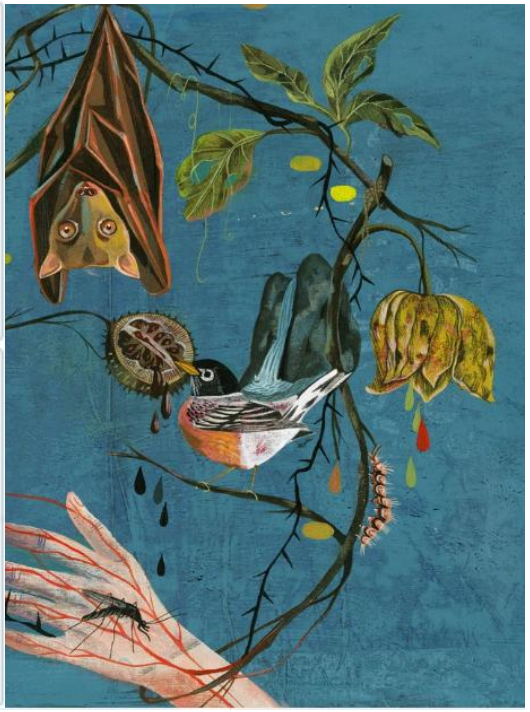
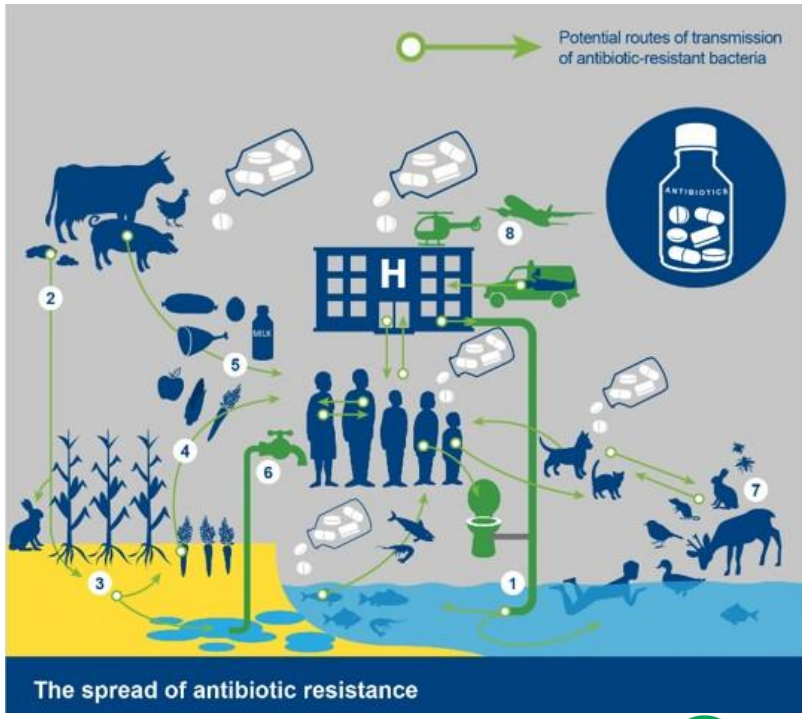
Defining priorities for wastewater-based pathogen surveillance

Environmental Surveillance & Disease Ecology





Antimicrobial Resistance



Vector-borne pathogens

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The ROCKEFELLER FOUNDATION

BWSSB

BBMP

Farah Ishtiaq, PhD

Principal Scientist

Tata Institute for Genetics and Society

ASIA PGI Webinar Series

23.07.2025

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National Centre for Biological Sciences
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Science for Society

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- TIGS is a unique initiative of the Tata Trusts founded in 2017, is a non-profit research institute that aspires to develop solutions to challenges in human health and agriculture.
- It's a program driven organization focused on societal relevance

Infectious Diseases

Environmental Surveillance & Disease Ecology

Antimicrobial Resistance

Vector Control

Rare Genetic Diseases

Diagnosis

Treatment

Carrier Screening

Crop Improvement

Mutation Breeding

Genome editing

Pest management

Facilities and Technology platforms

Insectary

Diagnostics Development

Therapeutics

- **Protein Therapeutics**
- **mRNA Therapeutics**
- **Cell-Based Therapeutics**

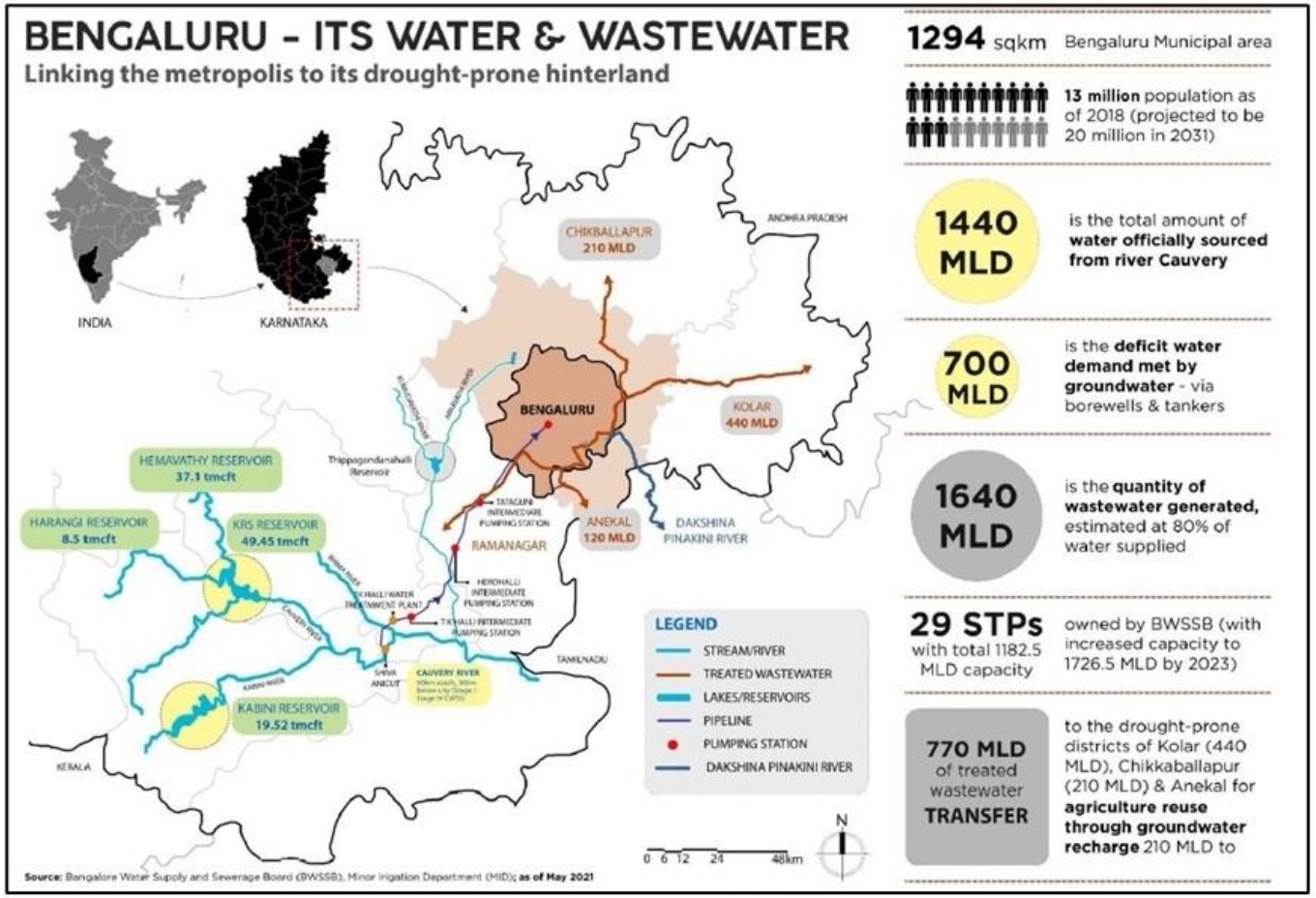
Community Engagement

Outreach

Stakeholder engagement

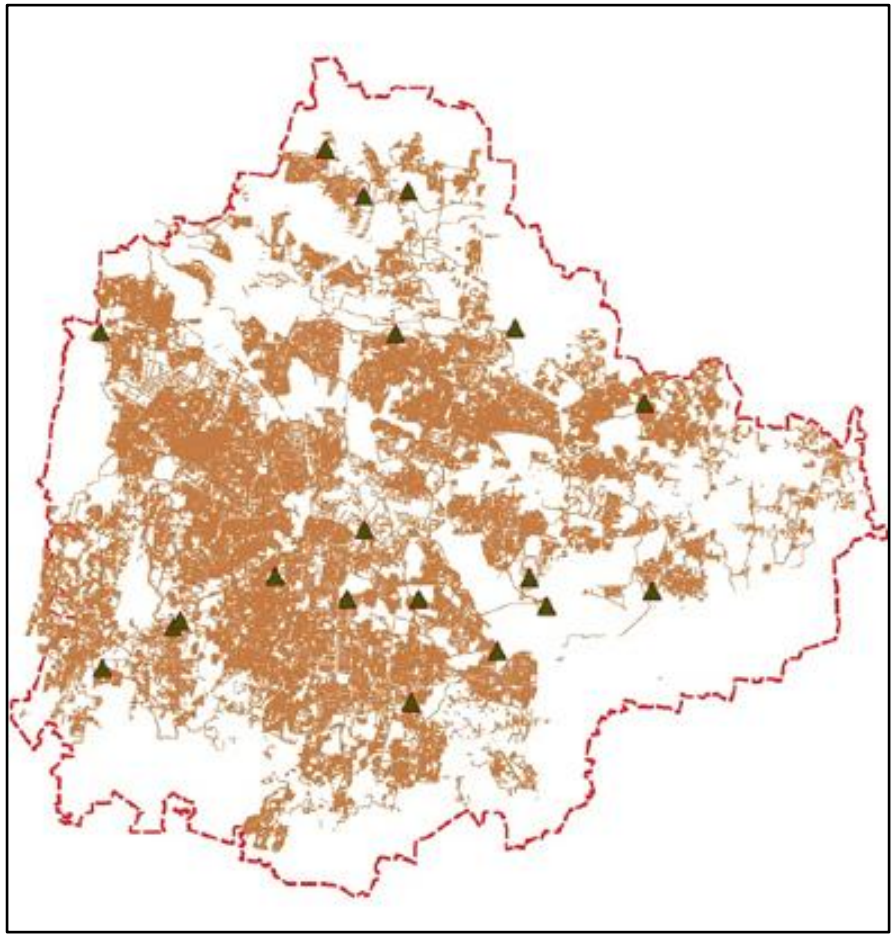
Policy research

Urban wastewaters are closely connected to climate change, diversity and health



Map showing the water management (inflow and outflow) of Bengaluru.

Bengaluru sewage network serving ~70% of the population



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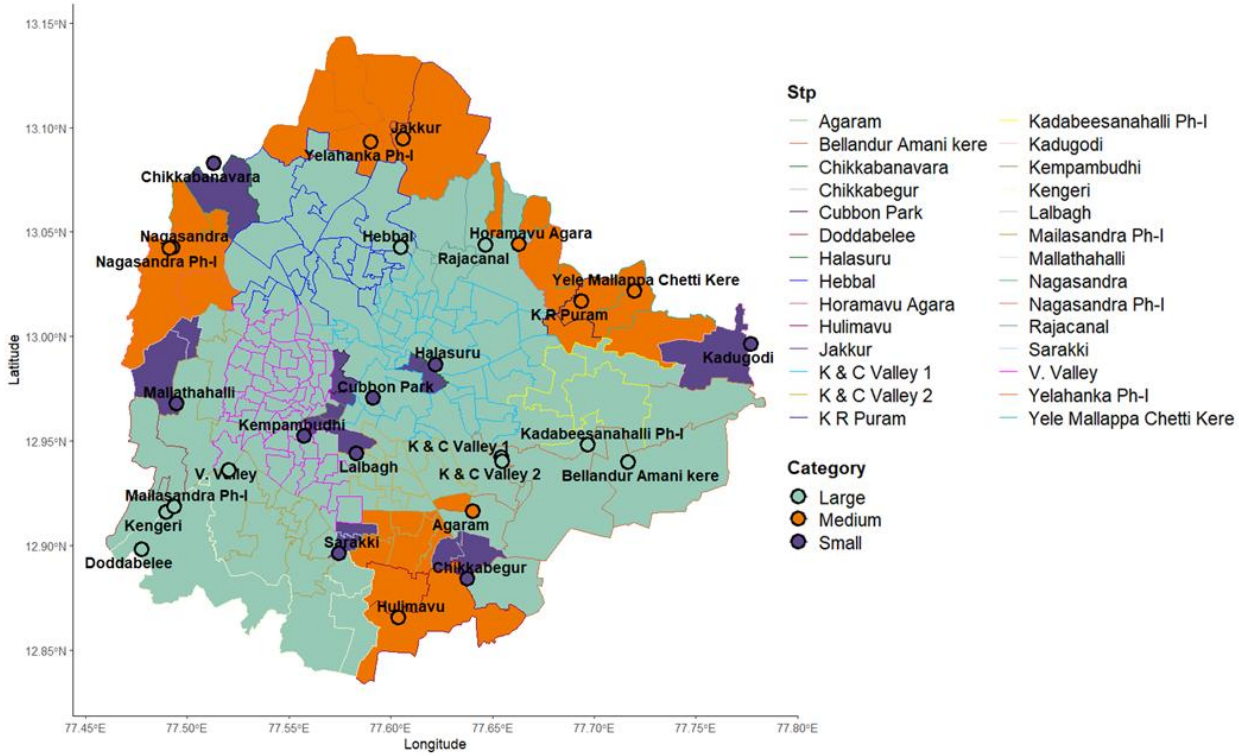
Goals for Environmental Surveillance

a) to establish ES as an epidemiological tool for predicting and pre-emptively responding to disease outbreaks.

b) to develop an adaptable protocol for identifying ES site locations for public health action.

c) to create protocols for safe reuse of treated water through evidence-based policy looking at both pathogens and emerging contaminants.

Location of sewage treatment plants under SARS-CoV-2 surveillance in Bengaluru since August 2021



eight **small sizes** (serving 10,000-60,000 population),
nine **medium sizes** (serving 100,000 – 350,000 population)
nine **large sizes** (serving 400,000 to 2,480,000 population)

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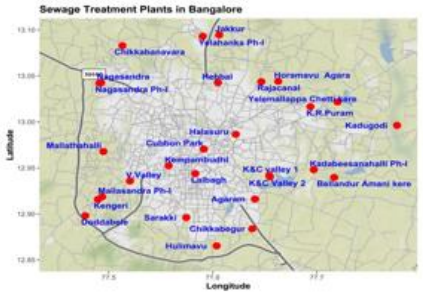
Integrating human and animal health with surveillance and disease ecology in a global urban center

BeST CLUSTER
BENGALURU SCIENCE AND TECHNOLOGY (BeST) CLUSTER

Phase 1: Wastewater surveillance as a complementary tool for monitoring SARS-COV-2 spread and evolution, emergence of new variants

Office of the Principal Scientific Adviser to the Government of India
AN INITIATIVE BY THE OFFICE OF THE PRINCIPAL SCIENTIFIC ADVISER TO THE GOVERNMENT OF INDIA

Wastewater-based Epidemiology of SARS-CoV-2 in Bengaluru

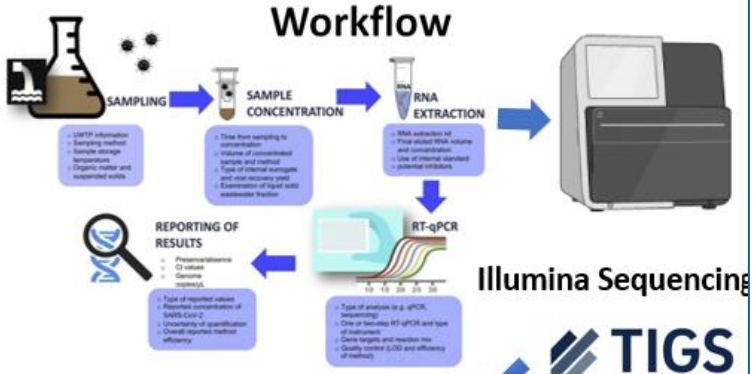


Sewage Treatment Plants in Bangalore

Sewage Treatment Plant

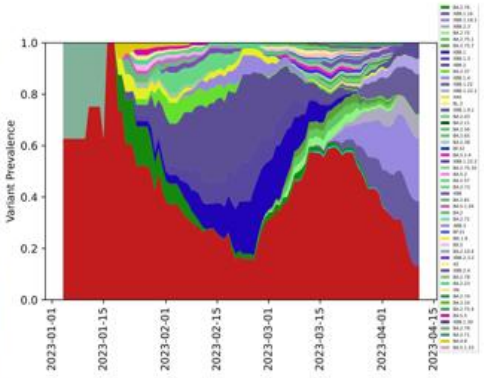
Inlet

Outlet

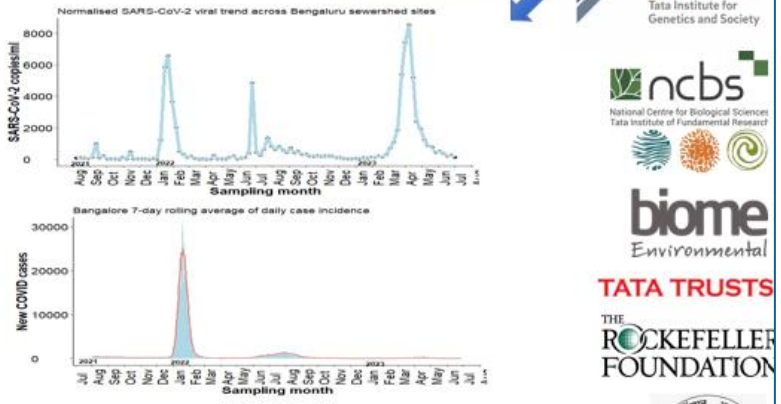


Illumina Sequencing

Sense-making workshop with stakeholders



Variant Prevalence



Temporal Dynamics of Normalized SARS-CoV-2 Viral load in Wastewater

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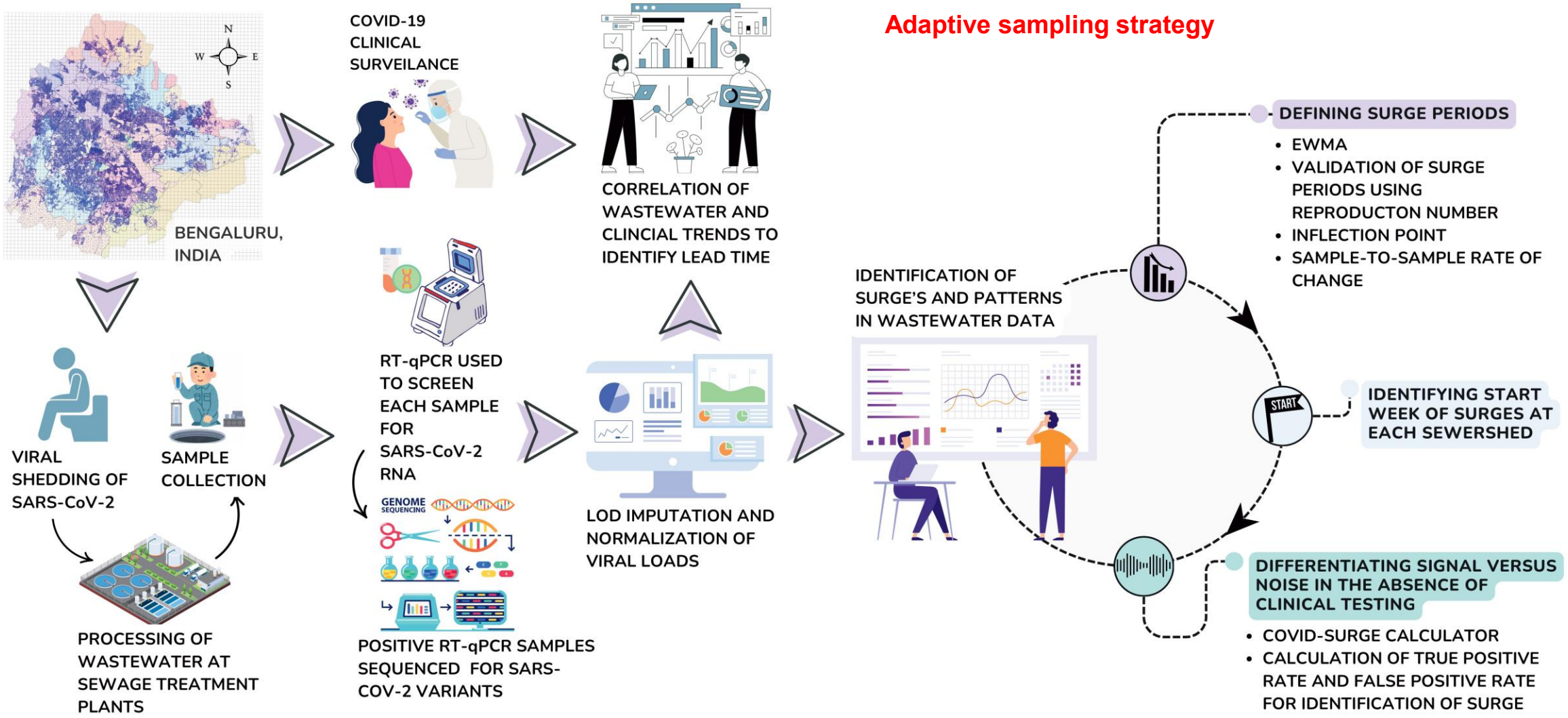
To pilot a convergent one-health approach that facilitates decision making for city administration.

Key elements:

- City wide data collection
- Data collation
- Data analysis
- Evidence-based decision making

- Interdisciplinary teams to work together to build Bengaluru City One Health Cell.

Identifying bellwether sewershed sites for sustainable disease surveillance in low-resource settings



Daniel R, Subash KK, N Daroch, S Ganesan, F Mozaffer, Vishwanath S, LS Shashidhara, R Mishra, **F Ishtiaq** (2025). Identifying bellwether sewershed sites for sustainable disease surveillance in Bengaluru-a longitudinal study. Lancet Regional Health SE Asia. Volume 39, August 2025, 100619. https://doi.org/10.1016/j.lansea.2025.100619

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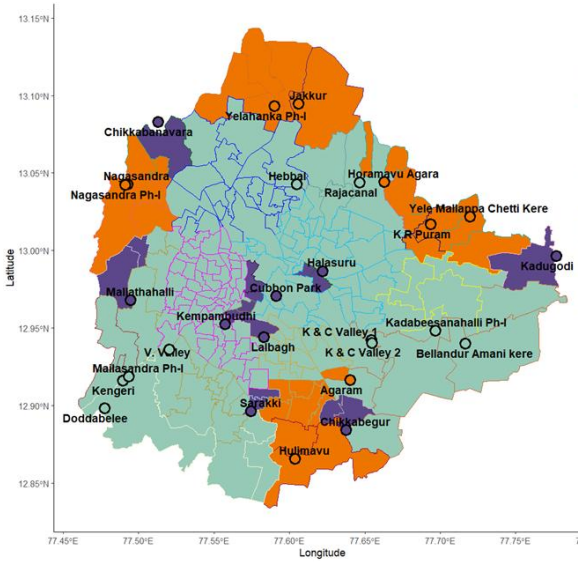
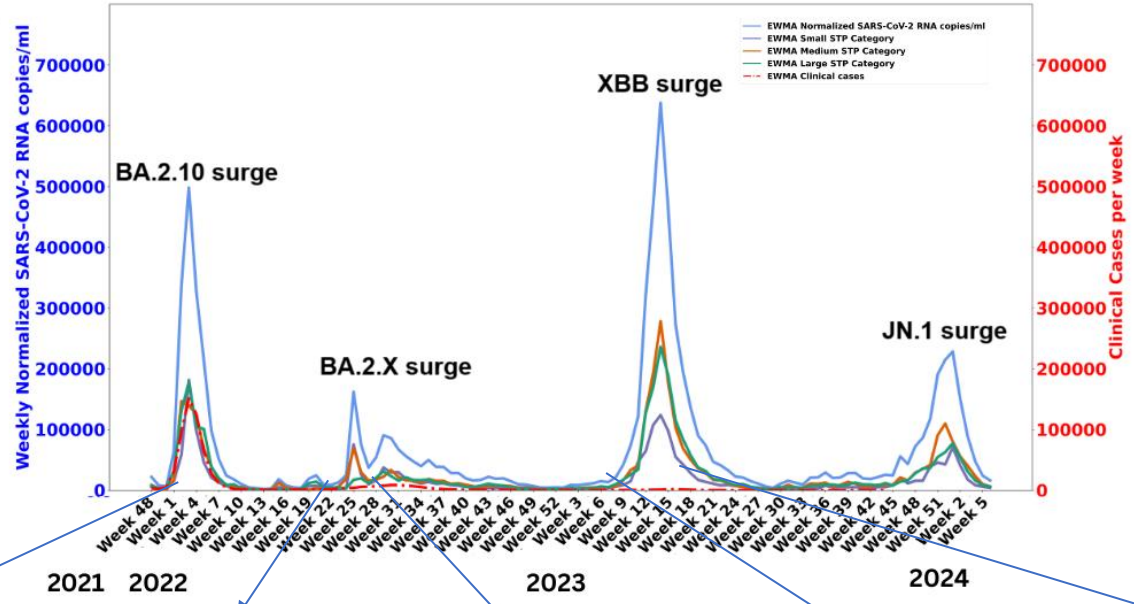
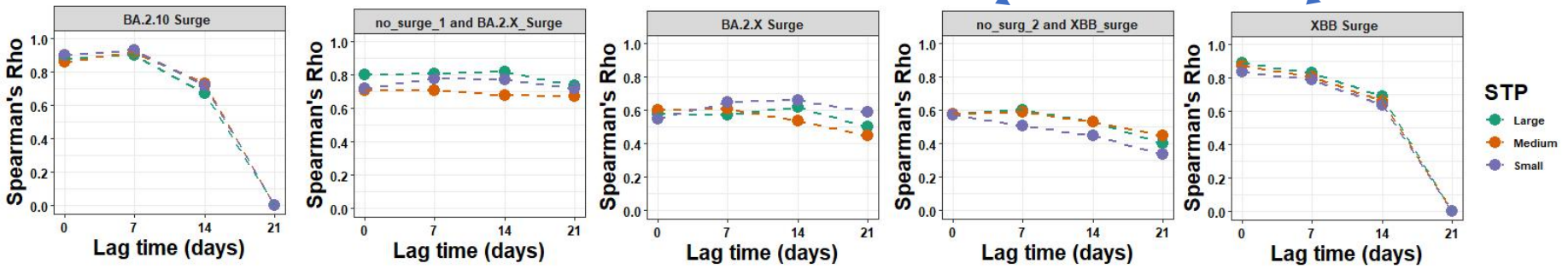
Comparison of viral load in sewage and COVID-19 cases in Bengaluru







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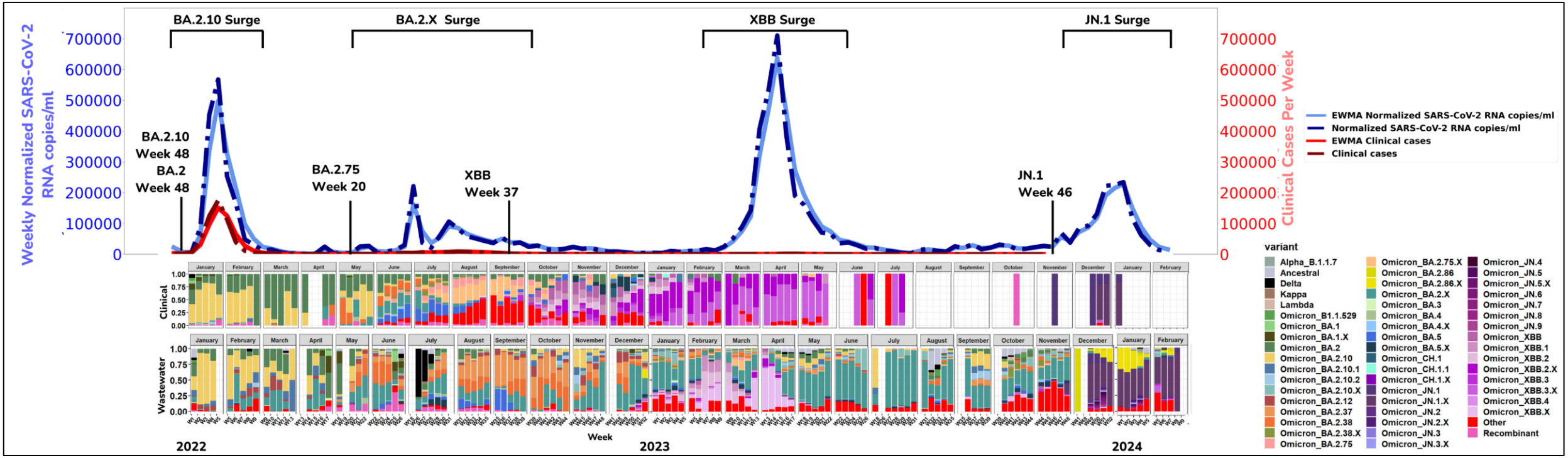
Genomic Surveillance: Targeted Next-generation sequencing (t-NGS)



Freyja pipeline used for wastewater analysis

Daniel R, Subash KK, N Daroch, S Ganesan, F Mozaffer, Vishwanath S, LS Shashidhara, R Mishra, **F Ishtiaq**[*] (2025). Identifying bellwether sewershed sites for sustainable disease surveillance in Bengaluru-a longitudinal study. Lancet Regional Health SE Asia. Volume 39, August 2025, 100619. https://doi.org/10.1016/j.lansea.2025.100619

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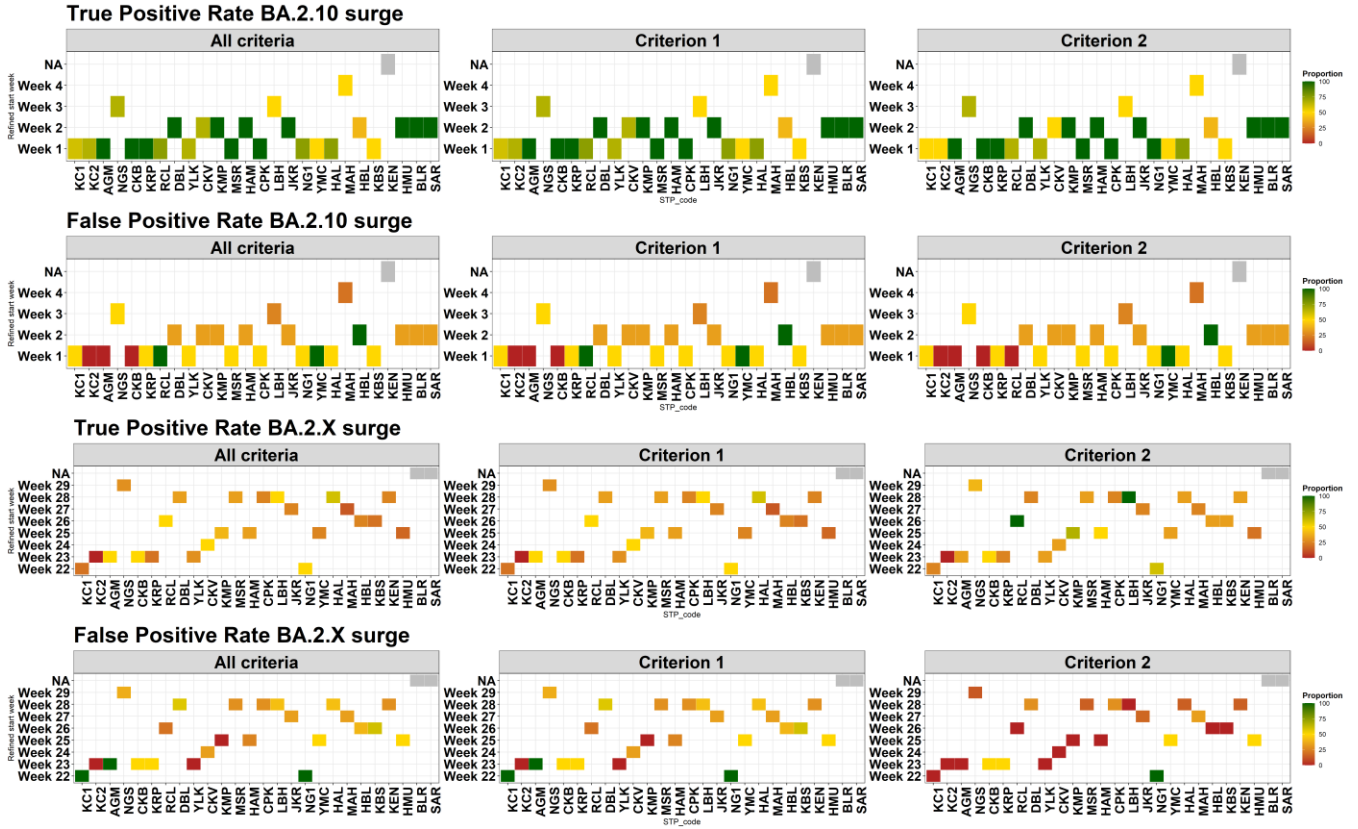
COVID-SURGE calculator (excel-based) to identify ‘Bellwether’ sewershed sites

The logical criteria applied to the COVID-SURGE algorithm to distinguish signal (sustained surges) from noise (sample-to-sample variations) in wastewater data.

Criterion 1	Was the current wastewater sample’s viral load higher than any other sample measured over the last four weeks?
Criterion 2.1	Did the viral load of the current sample represent a 100% increase or more from the previous sample?
Criterion 2.2	Did the viral load of the current sample represent a percent increase that was higher than any other samples observed in the last four weeks?
Criterion 3	Did the wastewater sample’s viral load become detectable after four continuous weeks of viral loads below the limit of detection?
Samples Flagged as community-level surges if any of the combinations of criteria were met:	Criteria 1 and 2.1 *OR* Criteria 1 and 2.2 *OR* Criteria 3



Keshaviah, A. *et al.* Separating signal from noise in wastewater data: An algorithm to identify community-level COVID-19 surges in real time. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(31), e2216021120. (2023).

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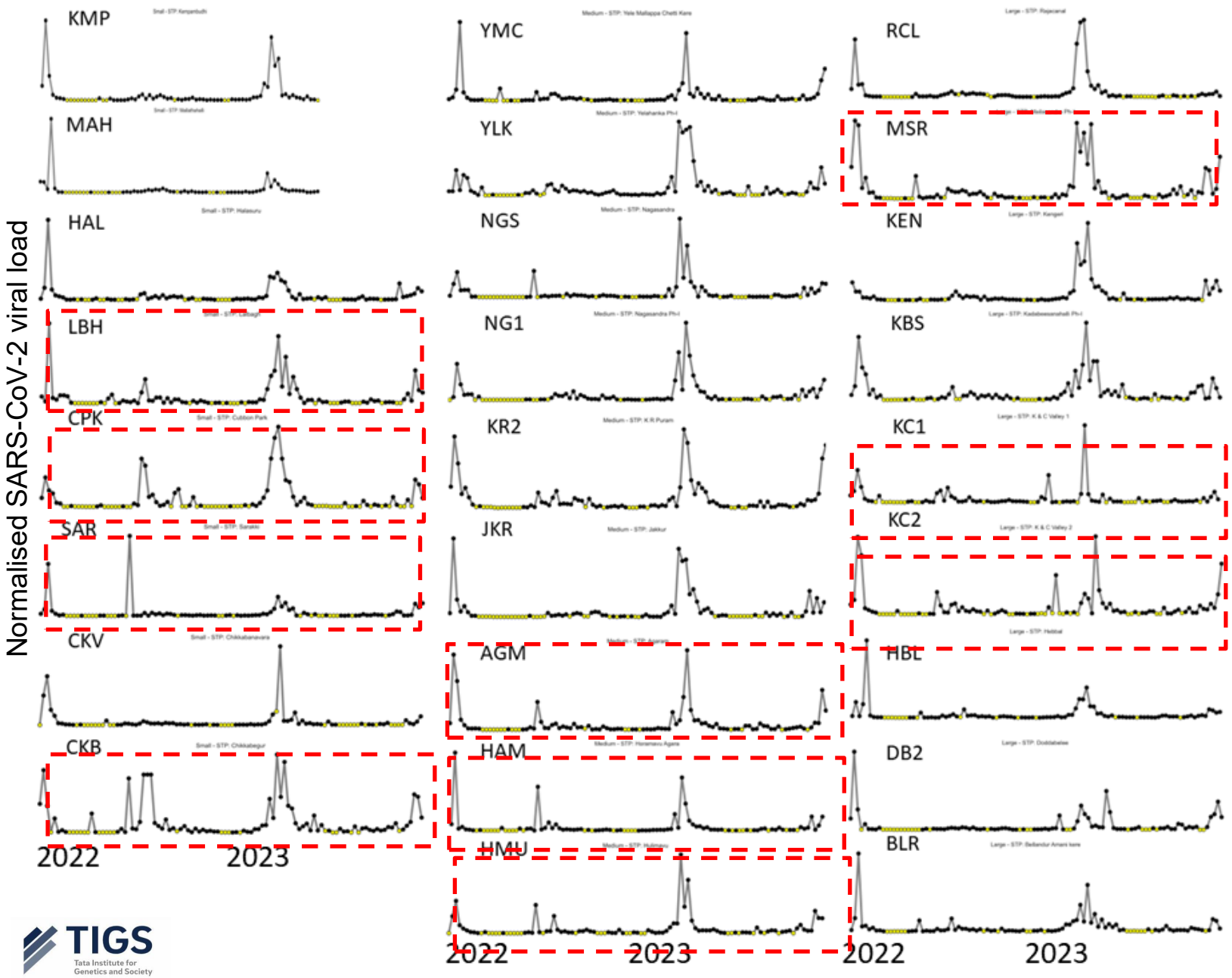

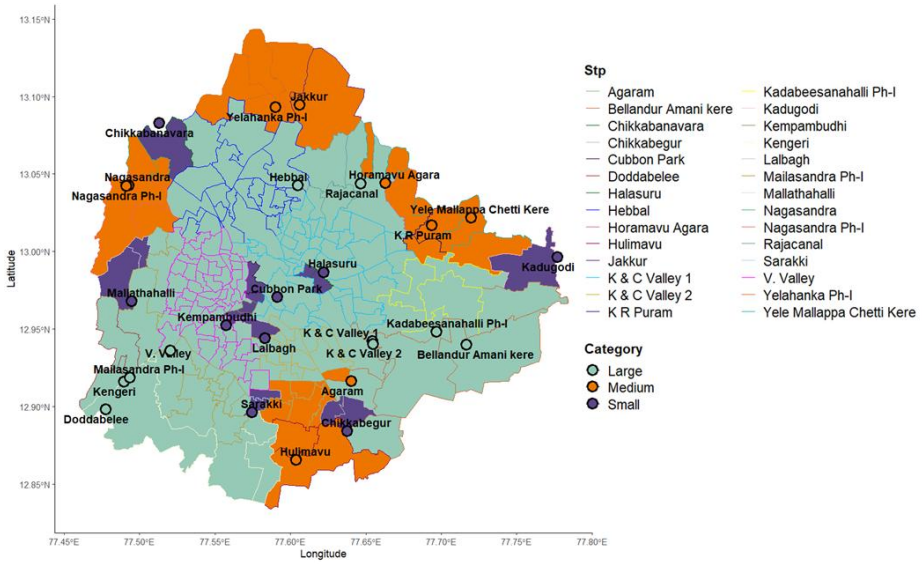

SARS-CoV-2 viral trend plots from January 2022 to December 2023 for each sewershed site

(Concentrations below the limit of detection are yellow points and those above the limit of detection are black points)

Daniel R, Subash KK, N Daroch, S Ganesan, F Mozaffer, Vishwanath S, LS Shashidhara, R Mishra, **F Ishtiaq** (2025). Identifying bellwether sewershed sites for sustainable disease surveillance in Bengaluru-a longitudinal study. Lancet Regional Health SE Asia. Volume 39, August 2025, 100619. https://doi.org/10.1016/j.lansea.2025.100619

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nature > nature india > comment > article

COMMENT | 15 December 2022

India must scale up wastewater analysis for health surveillance

Wastewater testing helped identify COVID-19 hotspots almost a couple of weeks before clinical data could

Farah Ishtiaq

Why environmental surveillance for avian influenza is vital

Birds infected with avian influenza virus shed large quantities of virus in their faeces as well as in their saliva and nasal secretions for about a week

Active surveillance of poultry imperative

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The Lancet Regional Health - Southeast Asia
Volume 11, April 2023, 100151

Articles

SARS-CoV-2 infection dynamics and genomic surveillance to detect variants in wastewater – a longitudinal study in Bengaluru, India

Sanjay Lamba [a,d], Sutharsan Ganesan [a,d], Namrta Daroch [a], Kiran Paul [a], Soumya Gopal Joshi [a], Darshan Sreenivas [b], Annamalai Nataraj [a], Vishwanath Srikantaiah [c], Rakesh Mishra [a], Uma Ramakrishnan [b], Farah Ishtiaq [a]

nature india

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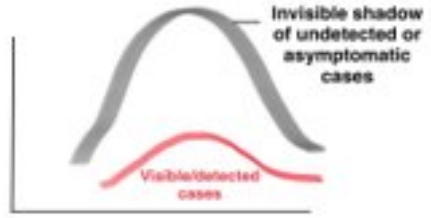
COMMENT | 15 June 2023

Ignoring wastewater is a wasted opportunity to improve disease response

As the COVID-19 emergency status fades, sewerage surveillance should be maintained

Farah Ishtiaq



Home > Features > A Bengaluru group is knee-deep in wastewater—looking for secrets on future diseases

Features | The FinePrint

A Bengaluru group is knee-deep in wastewater —looking for secrets on future diseases

Ecologist Farah Ishtiaq and her team are tracking viruses and diseases through Bangalore's underbelly. Th wastewater-based epidemiology project is catching attention.

SANDHYA RAMESH 1 March, 2023 11:42 am IST

ETPrime

Covid unlikely to overwhelm hospitals: Experts

By Teena Thacker, ET Bureau · Last Updated: May 04, 2023, 12:31 AM IST

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Synopsis
"In fact, waste water surveillance in Bengaluru has shown that we went through a silent outbreak. Infections happened, but hospitals didn't fill up," said Virologist Shahid Jameel of the University of Oxford. "This shows vaccines and prior infections protect and the virus is pretty much endemic."

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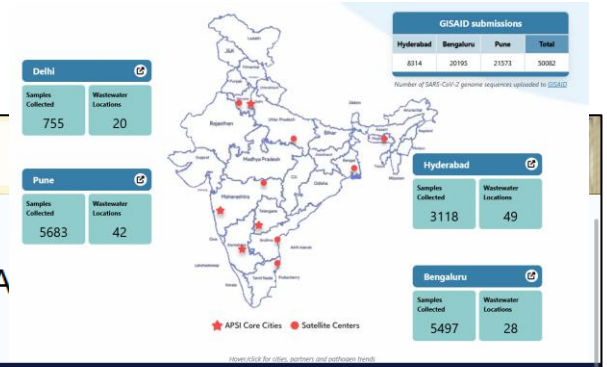
Guidelines and Standard Operating Procedures for ES

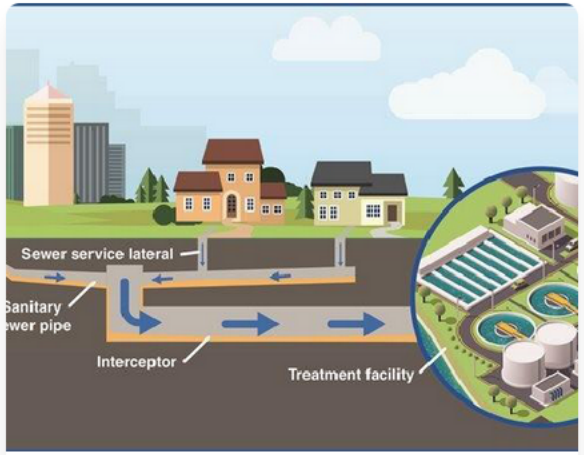


data.ccmb.res.in/apsi/protocol/

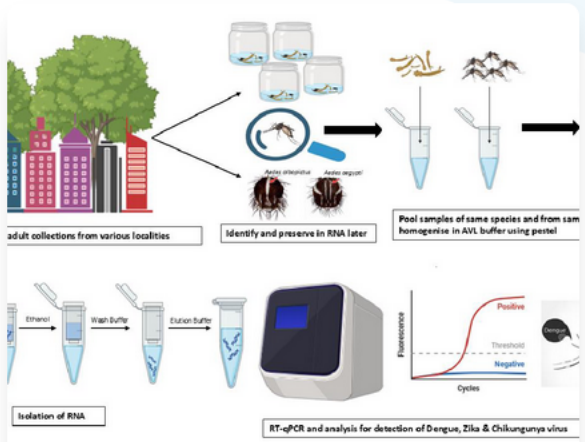
APSI
Alliance for Pathogen Surveillance Innovations -India

Home About Teams Publications Outreach Gallery Protocols A

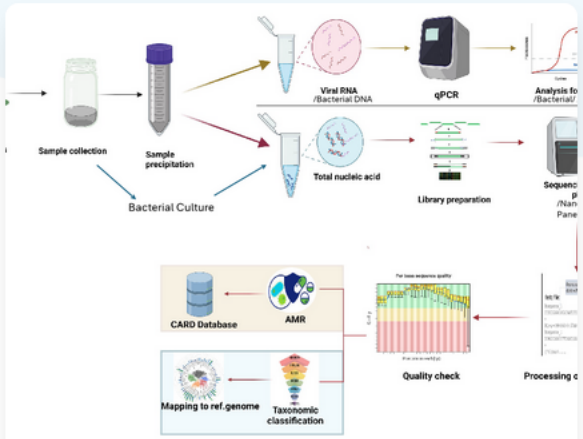
Standard Operating Procedures (SOPs) for conducting environmental surveillance of pathogens



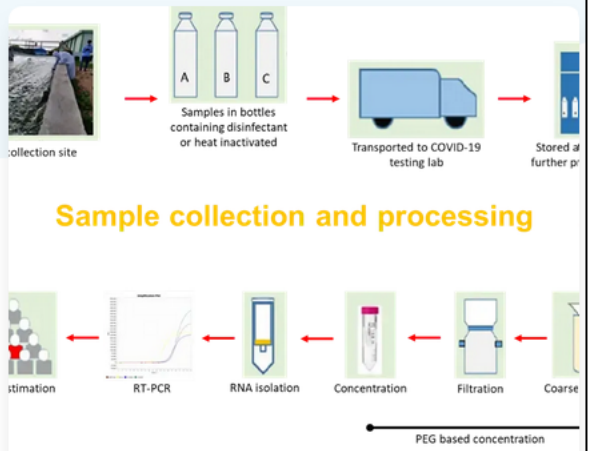
Guideline document for environmental surveillance site selection.pdf



SOP for detection of dengue and other arboviruses in Aedes mosquitoes.pdf

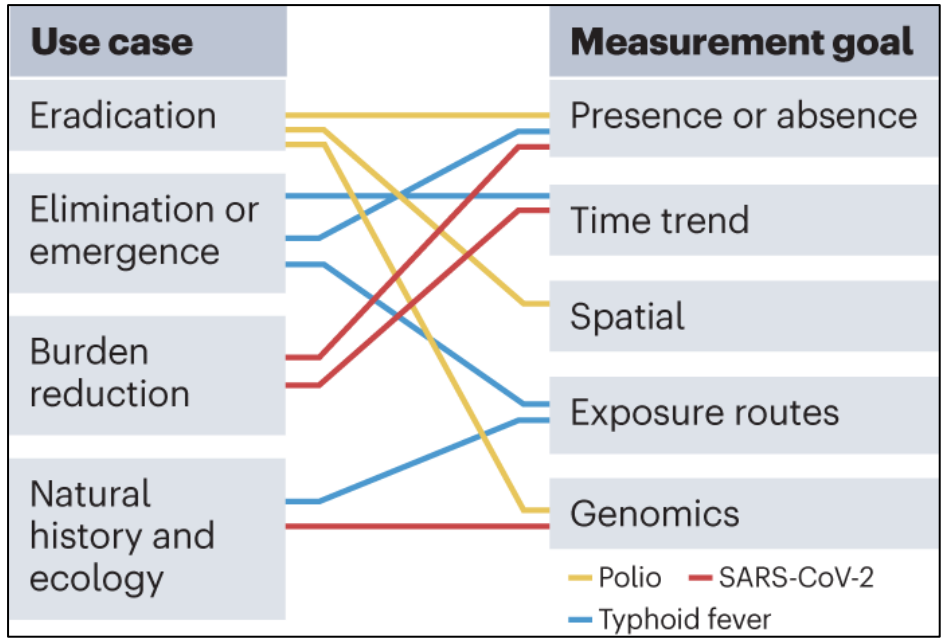


SOP for wastewater surveillance of pathogens contributing to AMR.pdf

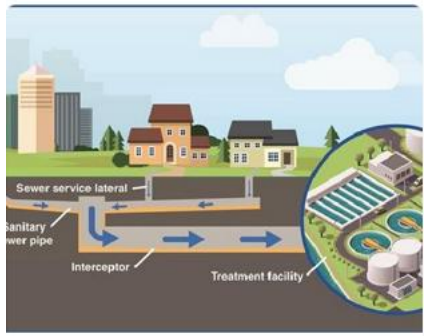


SOP for wastewater surveillance of viral pathogens.pdf

Defining priority pathogens



Shaw *et al*. *Nat Med* **29**, 2155–2157 (2023)



Guideline document for environmental surveillance site selection.pdf

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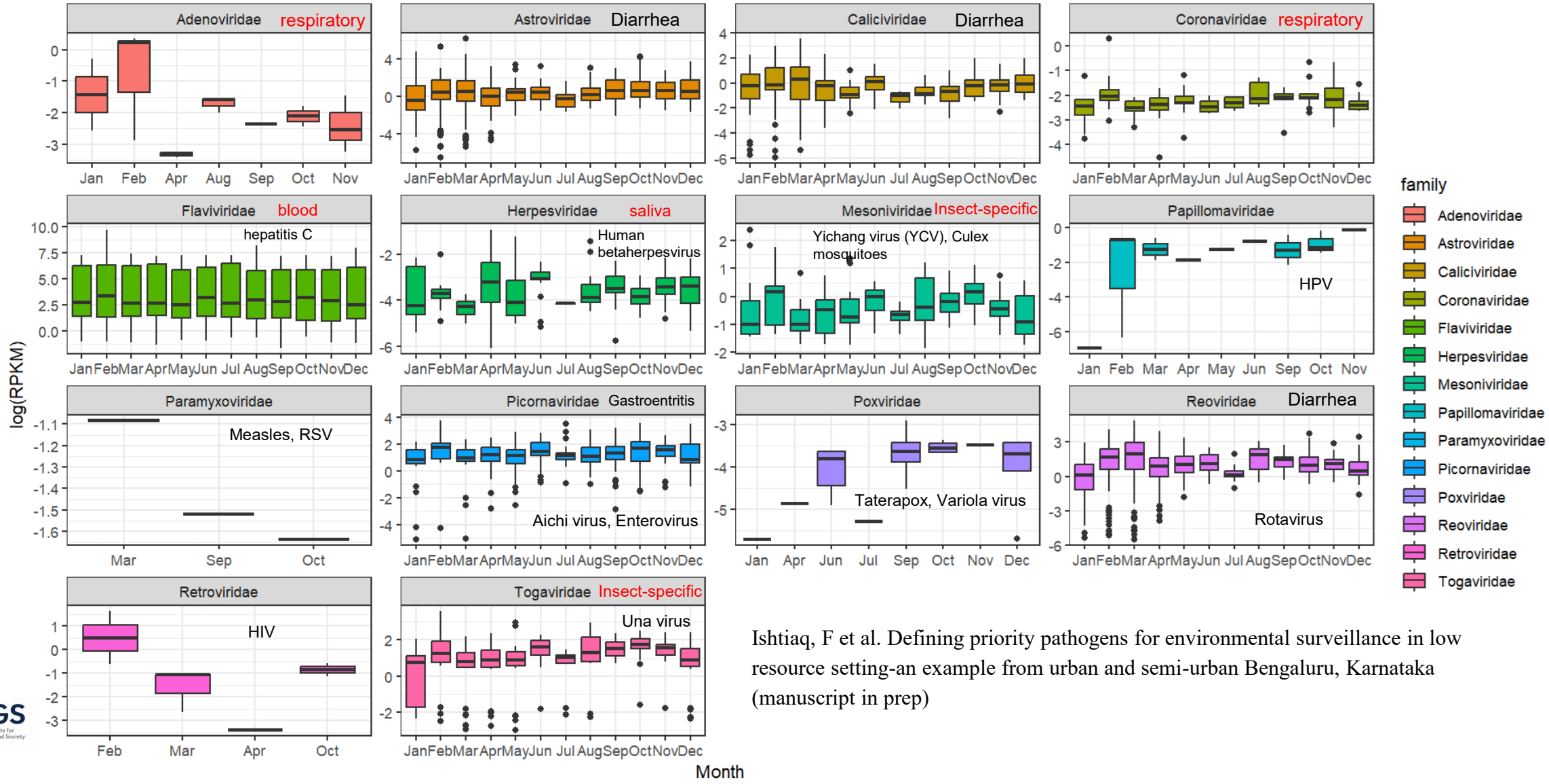
Table 1 illustrates the sample collection frequency, sample type, and intervention plan for each pathogen, molecular methods.

	USE CASE				MEASUREMENT GOALS					INTERVENTION				FREQUENCY			Sample type					Molecular testing		
Priority Pathogens	Eradication	Elimination or emergence	Burden reduction	Natural History and Ecology	Presence or absence	Time trend	Spatial distribution	Exposure routes	Genomics	Vaccination	Outbreak prepareness	Risk communication	R&D	Weekly	Bi-monthly	Monthly	Inlet	Outlet	Sludge	Satellite sites		Quantitative PCR	Digital Droplet PCR (ddPCR)	LC-MS/MS
Wildlife and Livestock Diseases																								
Avian Influenza		■		■	■	■	■	■	■		■	■			■		■			■		■	■	■
Mpox				■	■		■	■		■				■			■			■			■	■
Nipah				■	■		■	■			■	■				■	■			■				
Leptospira				■	■		■	■		■	■	■				■	■			■				
Human Diseases																								
Influenza A&B						■			■	■			■	■			■		■	■		■		
Resp. Syncytial Virus						■			■	■	■		■	■			■			■		■		
Cholera		■	■				■	■			■	■			■		■			■		■		
Hepatitis A&E		■	■				■		■	■		■		■			■			■		■		
Rotavirus							■	■		■	■	■		■			■			■		■		
SARS-CoV-2					■	■	■		■	■	■	■		■			■			■		■		
Dengue, Chikungunya, Zika		■	■	■		■	■	■	■	■			■	■			■		■	■		■	■	■
Norovirus							■		■		■	■		■			■			■		■		
Human Papiloma Virus (HPV)							■		■			■			■		■			■		■		
Candida auris infection							■	■	■		■	■	■			■	■	■	■	■		■		
Antimicrobial resistance																								
Antimicrobial resistance genes				■	■	■	■	■	■			■				■	■			■				
Microbial diversity				■	■	■	■	■	■			■				■	■			■				
Fungal diversity				■	■	■	■	■	■										■	■				
Emerging contaminants																								
Antibiotics				■	■	■	■	■			■	■				■	■		■	■				■
Antivirals				■	■	■	■	■			■	■				■	■	■	■	■				■
Antimalarials				■	■	■	■	■			■	■				■	■	■	■	■				■
Acetaminophen				■	■	■	■	■			■	■				■	■	■	■	■				■
Caffeine				■	■	■	■	■			■	■				■	■	■	■	■				■
Disinfectants				■	■	■	■	■			■	■				■	■	■	■	■				■
Heavy Metals			■		■		■	■				■	■			■	■	■	■	■				■

Defining priority pathogens for ES: Beyond SARS-CoV-2 (Aug 2021-Dec 2023)

Genomic Surveillance:
Unbiased metagenomics



Ishtiaq, F et al. Defining priority pathogens for environmental surveillance in low resource setting-an example from urban and semi-urban Bengaluru, Karnataka (manuscript in prep)

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Environmental Surveillance of Highly Pathogenic Avian Influenza Viruses

Farah Ishtiaq (TIGS, Bengaluru), Madhvi Joshi (GBRC, Gandhinagar), Aruna Panda & G. Umapathy (CCMB, Hyderabad), Jagdish M (ARTPARK) and LS Shashidhara (NCBS)

Current Surveillance System

- ❑ Passive
- ❑ Reactive

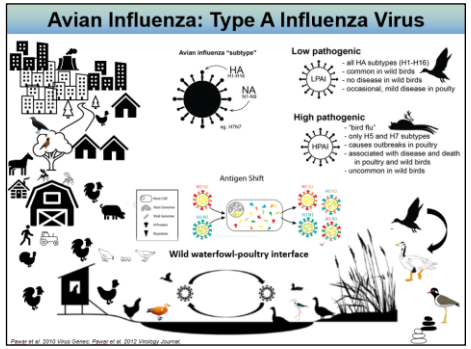
All testing is conducted by one government agency (NISHAD in collaboration with NIVEDI)

Low-cost, scalable approach for early warning: **Environmental Surveillance**

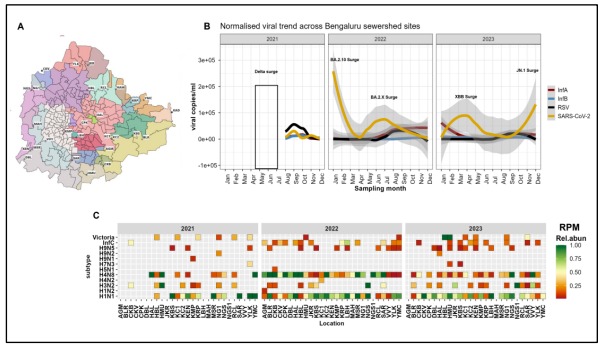
- ❑ Active
- ❑ Synergy from combined approaches

Non-invasive methods that can complement current methods with frequent, regular testing at scale

ES as One Health Tool

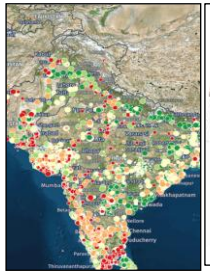


ES as Disease Sentinel

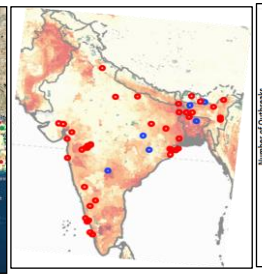


Ecological drivers of Avian Influenza

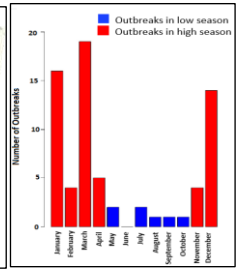
Wetlands surveyed for waterbird census



Poultry density distribution and avian influenza outbreaks



Seasonality of avian influenza outbreaks



Source: WOAH.org

ES Samples to cover Avian Influenza ecology

Water samples from rice fields with duck farms

Air Samples in Poultry farms

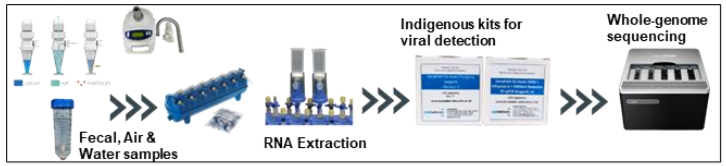
Fecal and run-off water samples from poultry and cattle farms

Urban sewage and water samples from wetlands, zoos and lakes with migratory birds

Samples

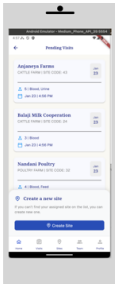
- ❑ Fecal
- ❑ Air
- ❑ Water

Sample processing

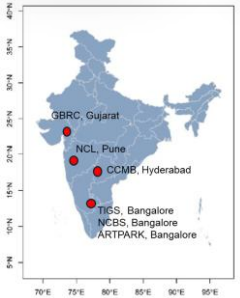


- All sampling methodologies validated by spiking known negative environmental samples with inactivated H5N1 virus.
- Limit of detection by quantitative RT-PCR kit = 3 copies/µl (EID50 2.14 X 10^4)

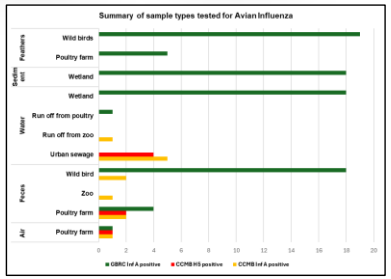
Real-time databases and analytical tools for predicting risk to human and animal health



The consortium



Results

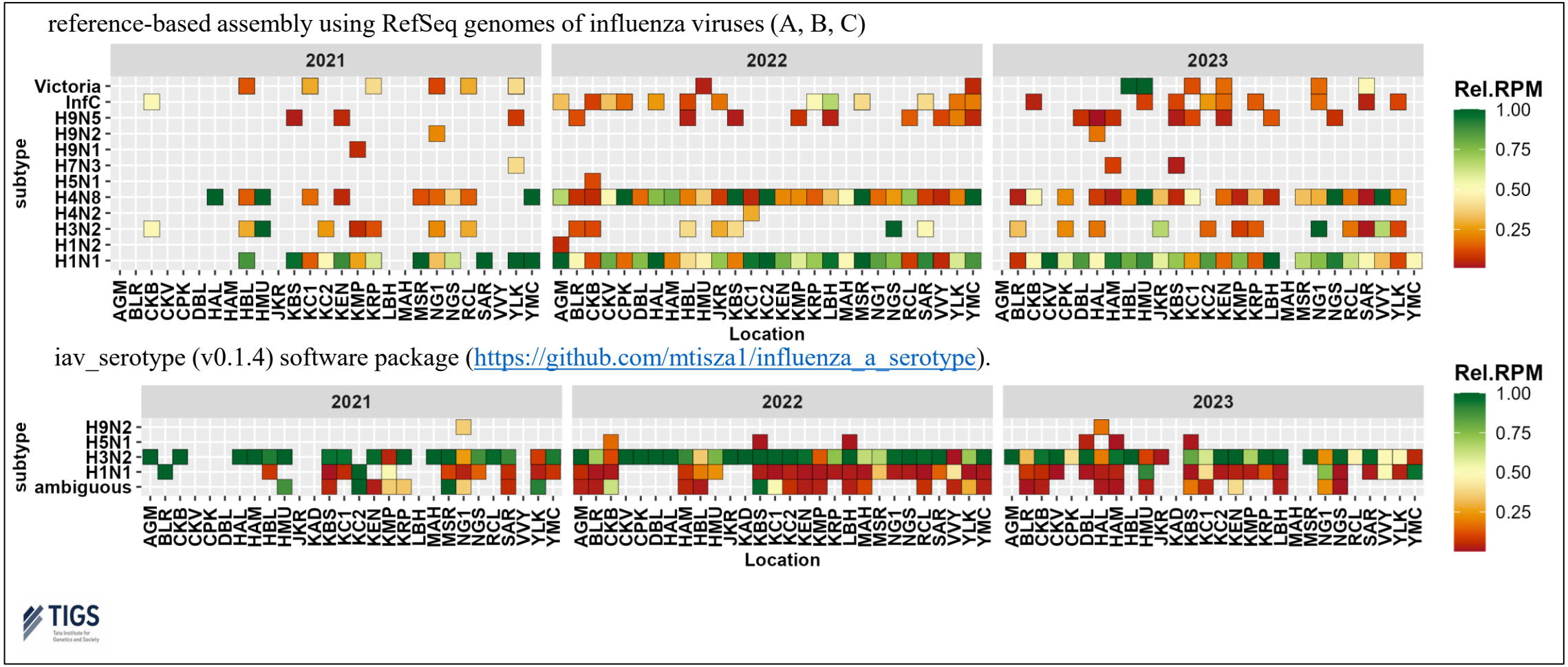


Future Plans

- Validating protocols for ES of avian influenza as early warning systems.
- **Develop low-cost, scalable, test-kits and tools for analyses of ES for avian influenza. Including targeted NGS**
- Integration of ES into existing surveillance systems which rely on culture-based methods for testing birds. ES would be a cost-effective and non-invasive method to assess the health of large populations at local, state and national levels.

Funding by:
Gates Foundation

Setting up protocols for environmental surveillance of Influenza viruses as an early warning system

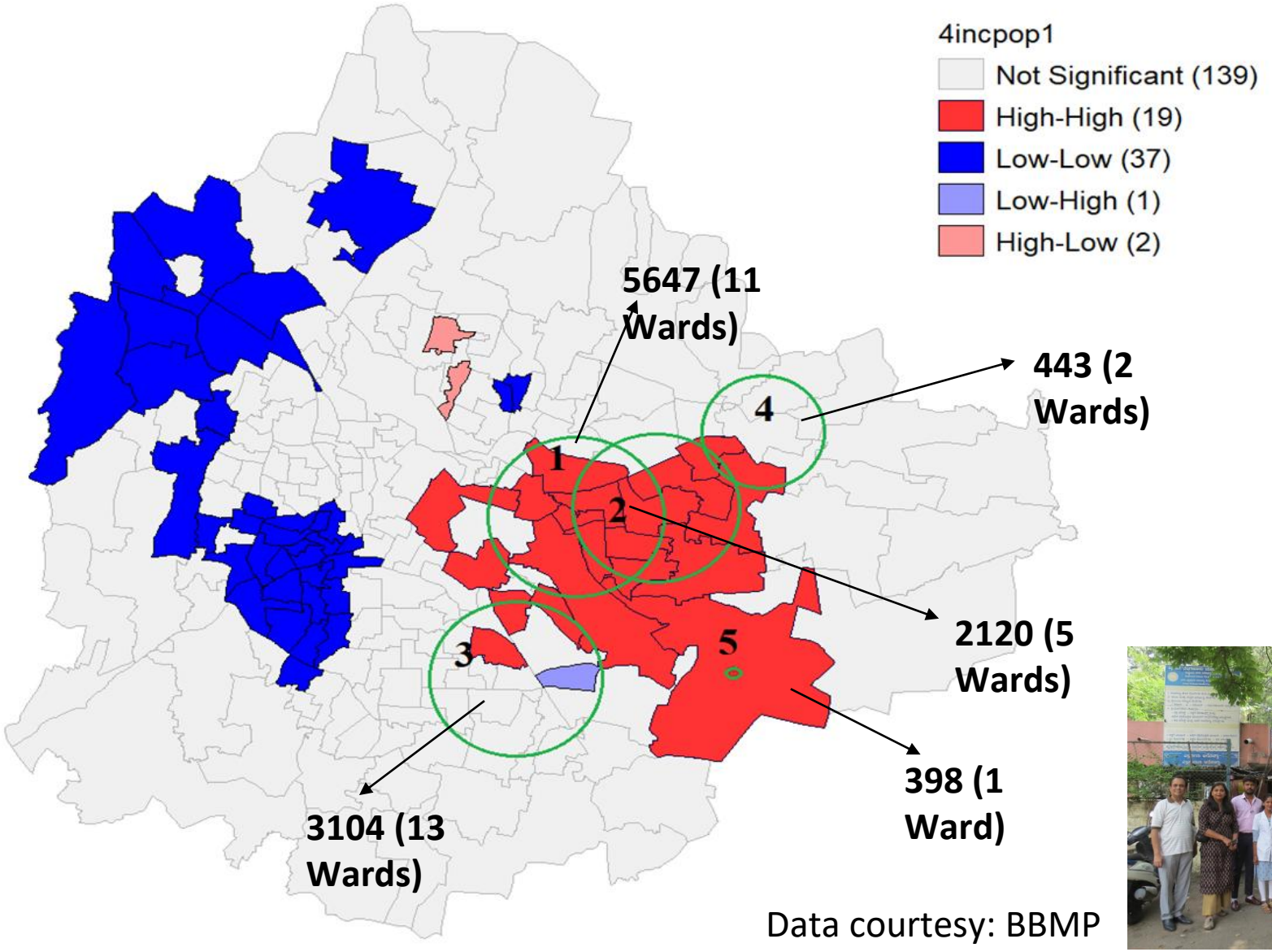


Daroch N, Subash KK, Vishwanath S, R Mishra, **F Ishtiaq**. Respiratory virus infection dynamics and genomic surveillance to detect seasonal influenza subtypes in wastewater: a longitudinal study in Bengaluru, India. doi: https://doi.org/10.1101/2025.01.13.25320458

Developing an early warning system to predict Dengue outbreaks In collaboration with municipal corporation

High Clusters

1. RR=7.81,LLR=5991.41
2. RR=5.13,LLR=1655.85



Data courtesy: BBMP

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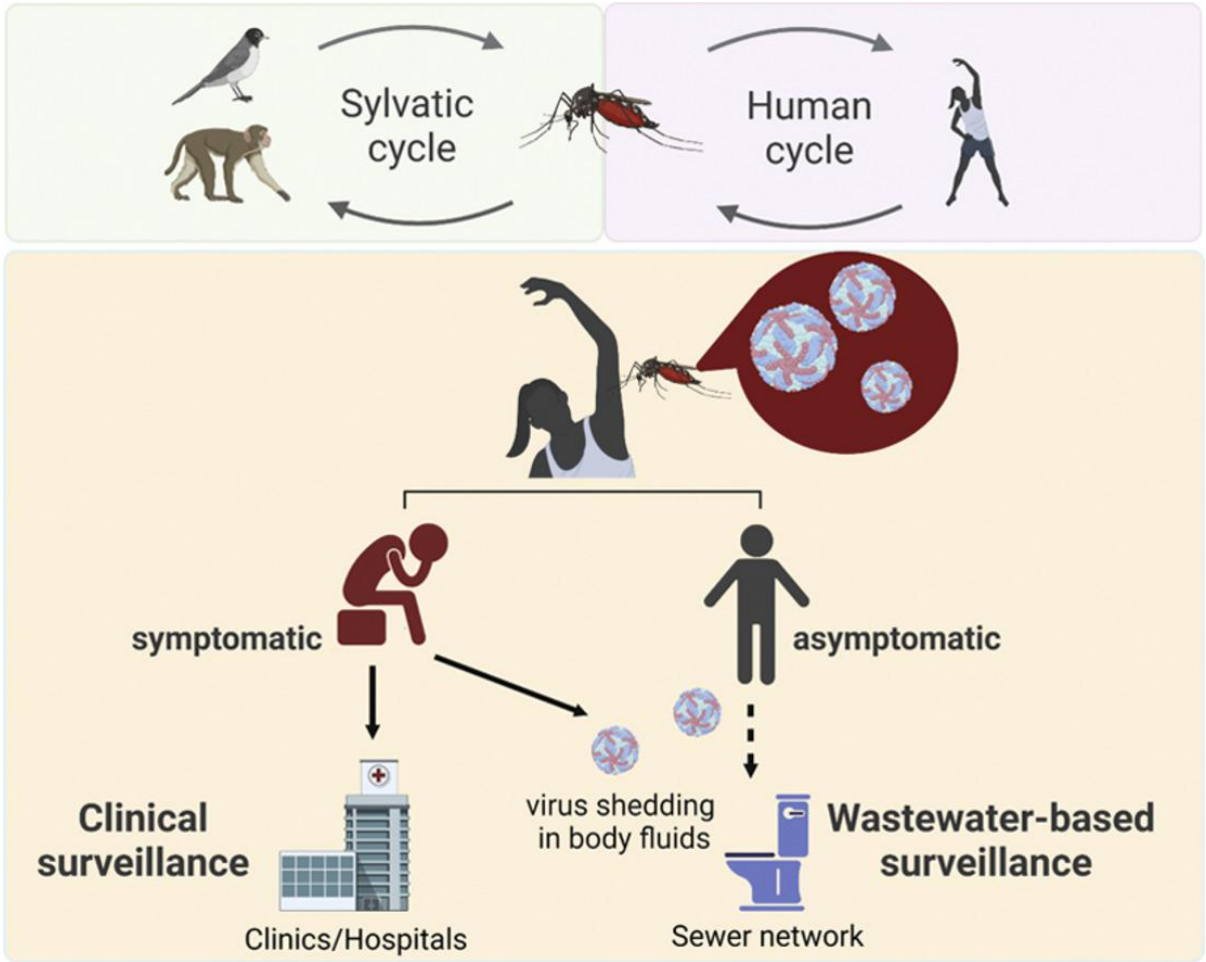
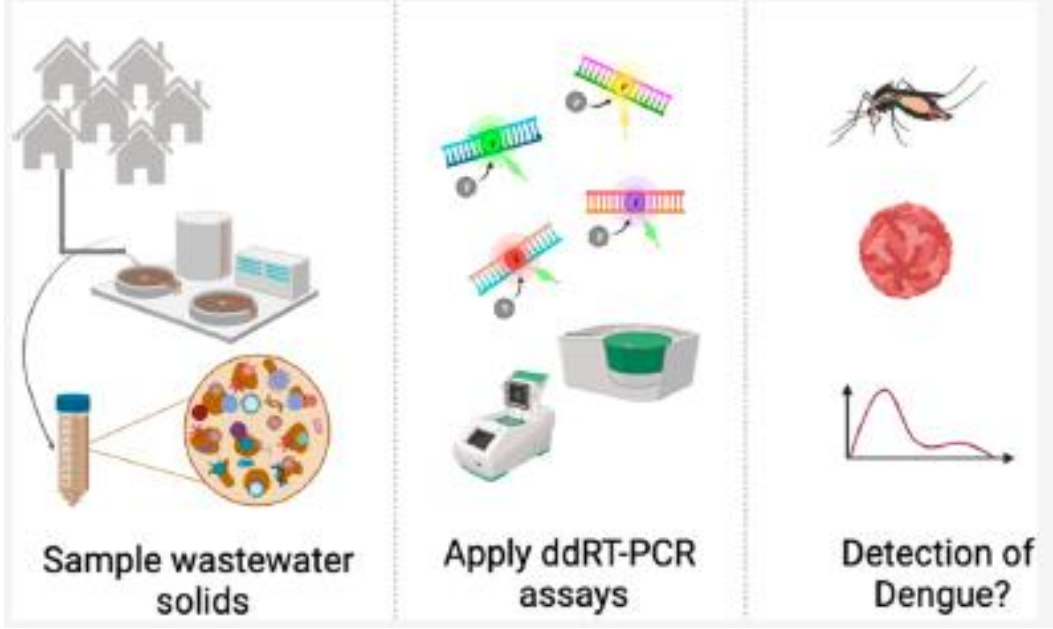
Wastewater surveillance for arboviral diseases

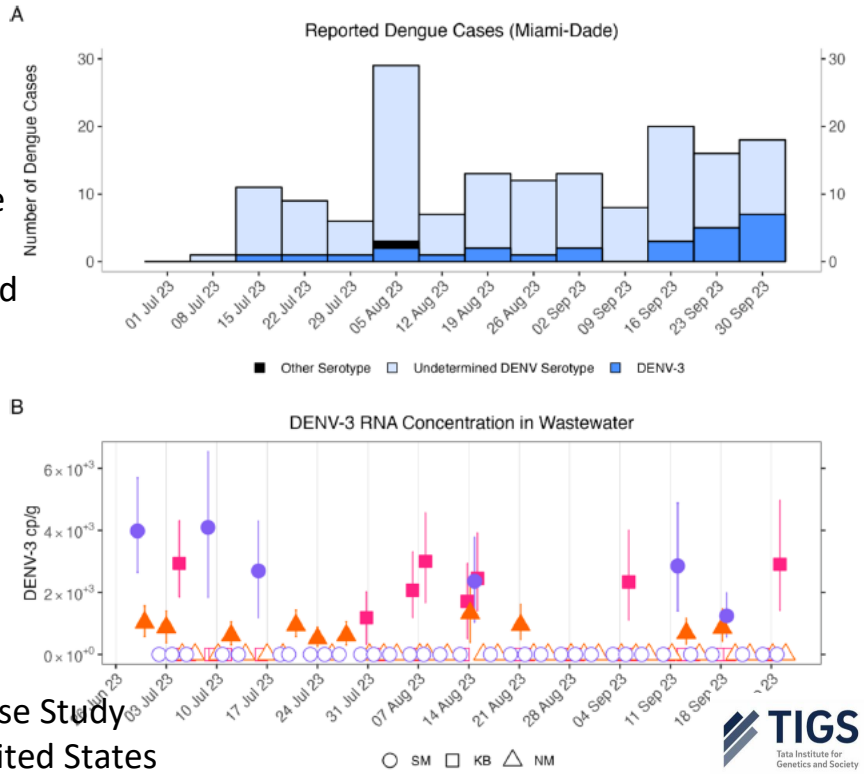


viral RNA can persist in sewage from 4C to 35 C up to 21 days

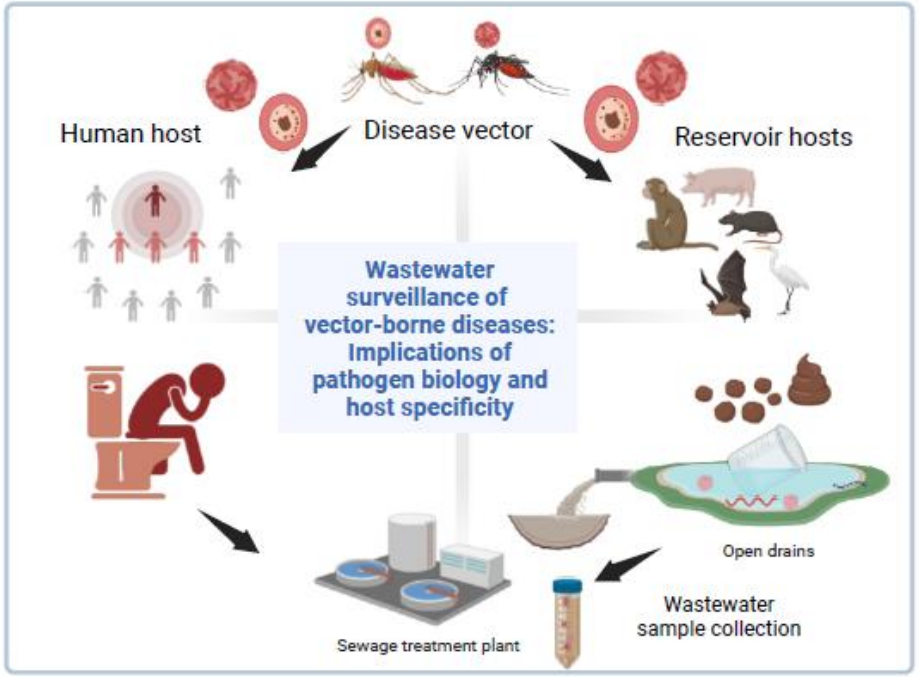
Lee *et al*. 2022

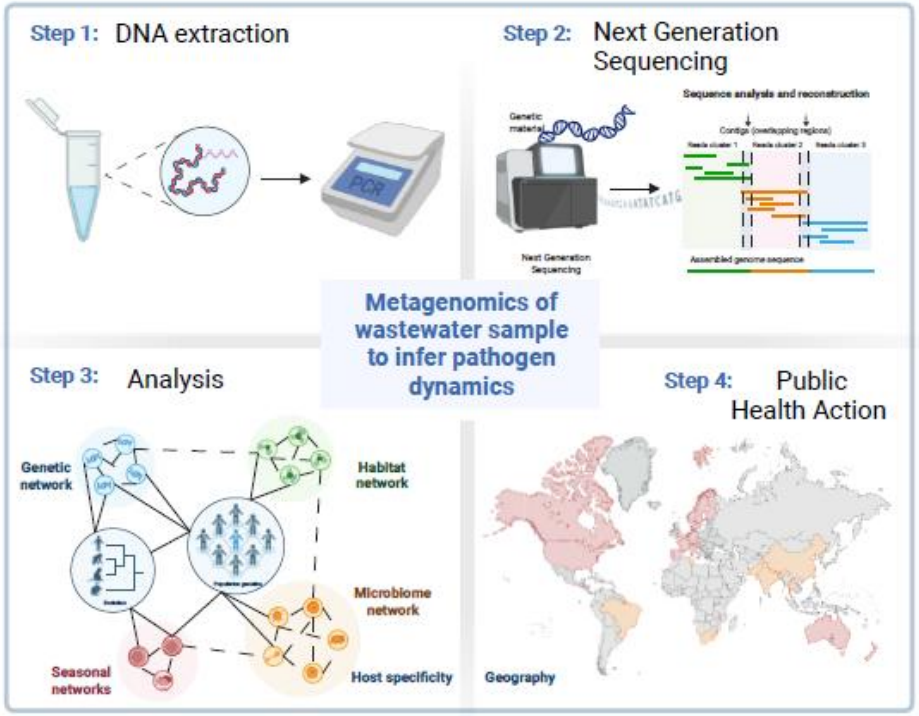


Wastewater detection of dengue virus RNA is possible with as few as 4.23 laboratory confirmed dengue cases per 1 million people



Wolfe *et al*. 2023 Case Study of Dengue in the United States





- **pathogen biology and host specificity,**
- **geographical context**
- **the availability of sewage networks for meaningful interventions.**

Dengue Environmental Wastewater Surveillance Summary

Sample Type	Non-treated wastewater sample		Wastewater settled solids
Processing methodology	PEG/NaCl concentration	PEG/NaCl/Dextran - Two phase seperation system *WHO protocol, 2003*	*Wolfe et al., 2024*
Sample count and its details	100 samples *(July – September 2022)*	100 samples July-September 2023, 2024	100 samples July-September 2023, 2024
Detection method	***RT-qPCR*** *(Pan-Dengue & Multiplex Dengue serotype specific RT-qPCR assay)*	***Droplet Digital PCR*** *Pan-Dengue*	***Droplet Digital PCR*** *Pan-Dengue*
Results	**Sample Control** – Pepper Mild Motile Virus (PMMoV) and **Extraction Control** (MS2 Bacteriophage RNA) - Positive for all sample Both Pan-Dengue and Multiplex Dengue serotype specific shows **negative** for all sample		

Ishtiaq F 2023 Trends in Parasitol.
https://doi.org/10.1016/j.pt.2023.12.005

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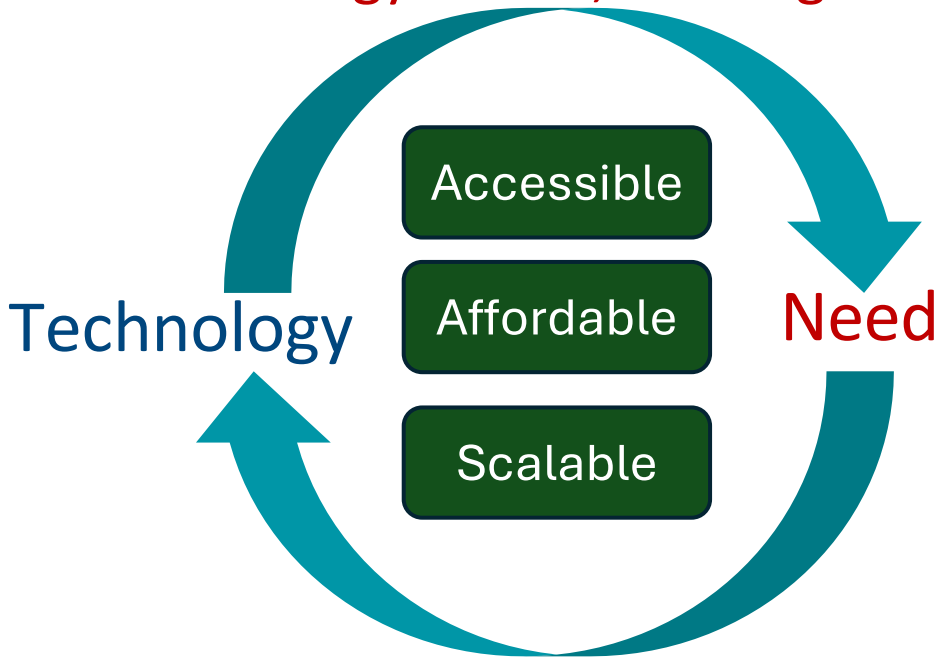
Diagnostics Development Platform

Need

- Infectious Diseases
- Rare Genetic Disorders
- Environmental Surveillance
- One Health

Diagnostics Development Platform

Technology-driven, need agnostic



Need-driven, Technology Agnostic

Technology

- CRISPR
- Real-time and DIgital PCR
- Omics NGS and metabolomics
- Rapid diagnostic test

Harvinder Kour Khera
Scientist

Mansi Malik
Scientist

Satyaprakash Pandey
Senior Scientist

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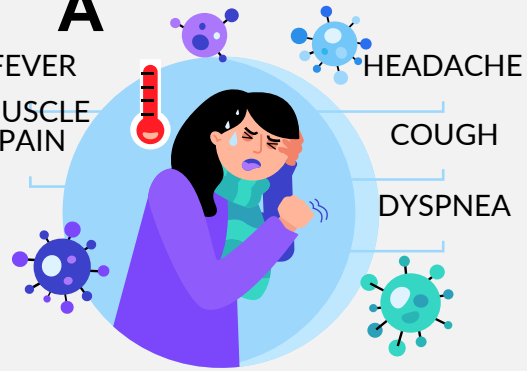
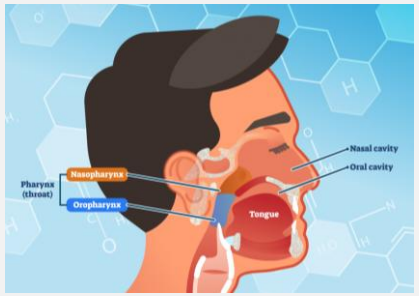
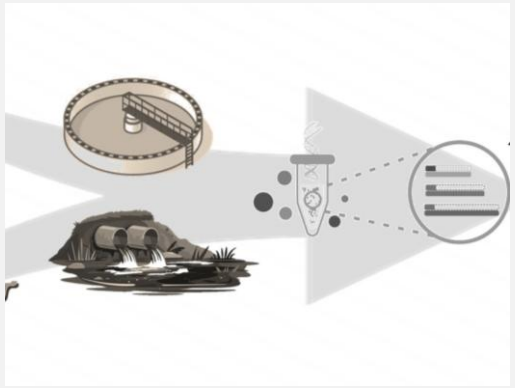
Multiplexed solutions for Respiratory panel and Cervical Screening

A

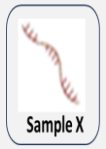


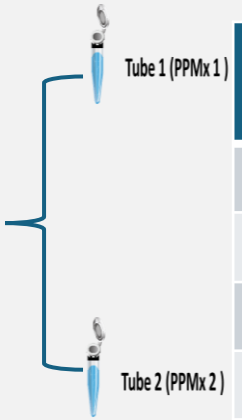


Nasopharyngeal Swabs



Wastewater sample

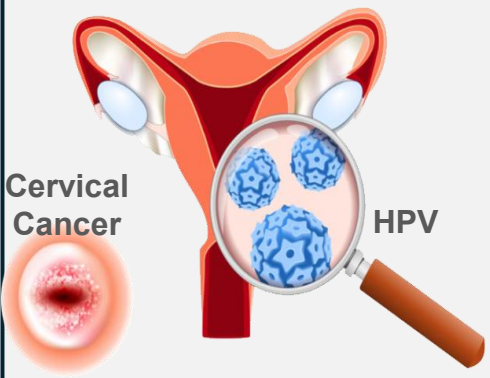




RespiFlu

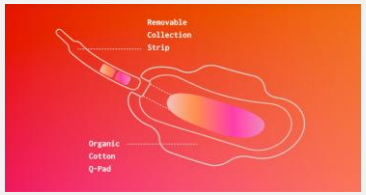
Target for PPMx Tube 1	Target for PPMx Tube 2
Influenza A	H1N1
Influenza B	RSV-AB
SARS-CoV-2	H3N2
IC	IC

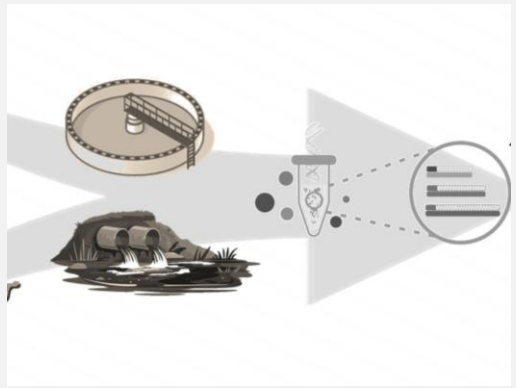
B



Cervical Swabs

Menstrual pad based





Wastewater sample

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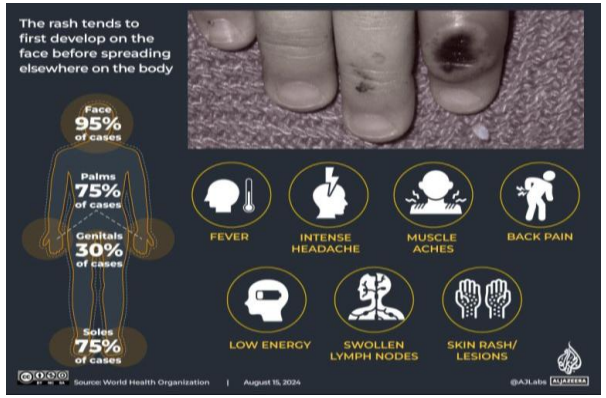
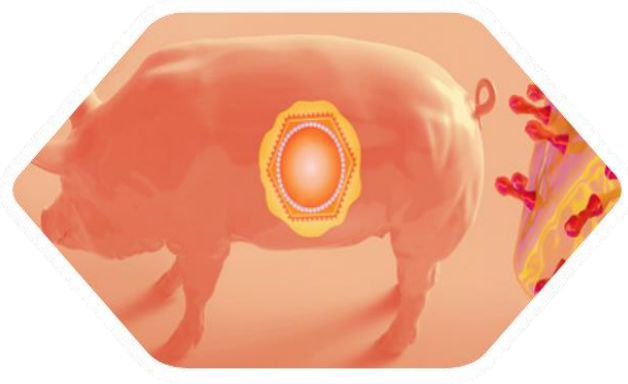
HPV genotyping

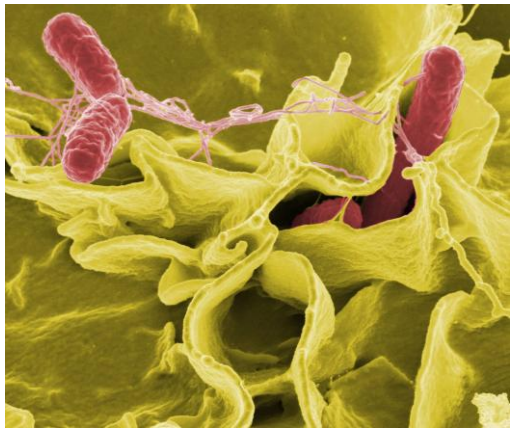
Target for PPMx 1	Target for PPMx 2	Target for PPMx 3	Target for PPMx 4
HPV 45	HPV 52	HPV 33	HPV 35
HPV 16	HPV 56	HPV 31	HPV 58
HPV 18	HPV 66	HPV 68	HPV 59
IC	HPV 39	HPV 73	HPV 51

Pandemic preparedness capability at TIGS

NEED → **< 45 days, Cost-effective, Interoperable** → **POTENTIAL SOLUTION**







H5N1	
LoD	1 copy/uL
Cross reactivity	None Observed
Turn Around time	<2.5 hours (from sample to results)

mpox	
LoD	5 copies/uL
Cross reactivity	None Observed
Turn Around time	<2.5 hours (from sample to results)

African Swine flu	
LoD	1 copy/uL
Cross reactivity	None Observed
Turn Around time	<2.5 hours (from sample to results)

Mycobacterium TB	
LoD	10 copies/uL
Cross reactivity	None Observed
Turn Around time	<2.5 hours (from sample to results)

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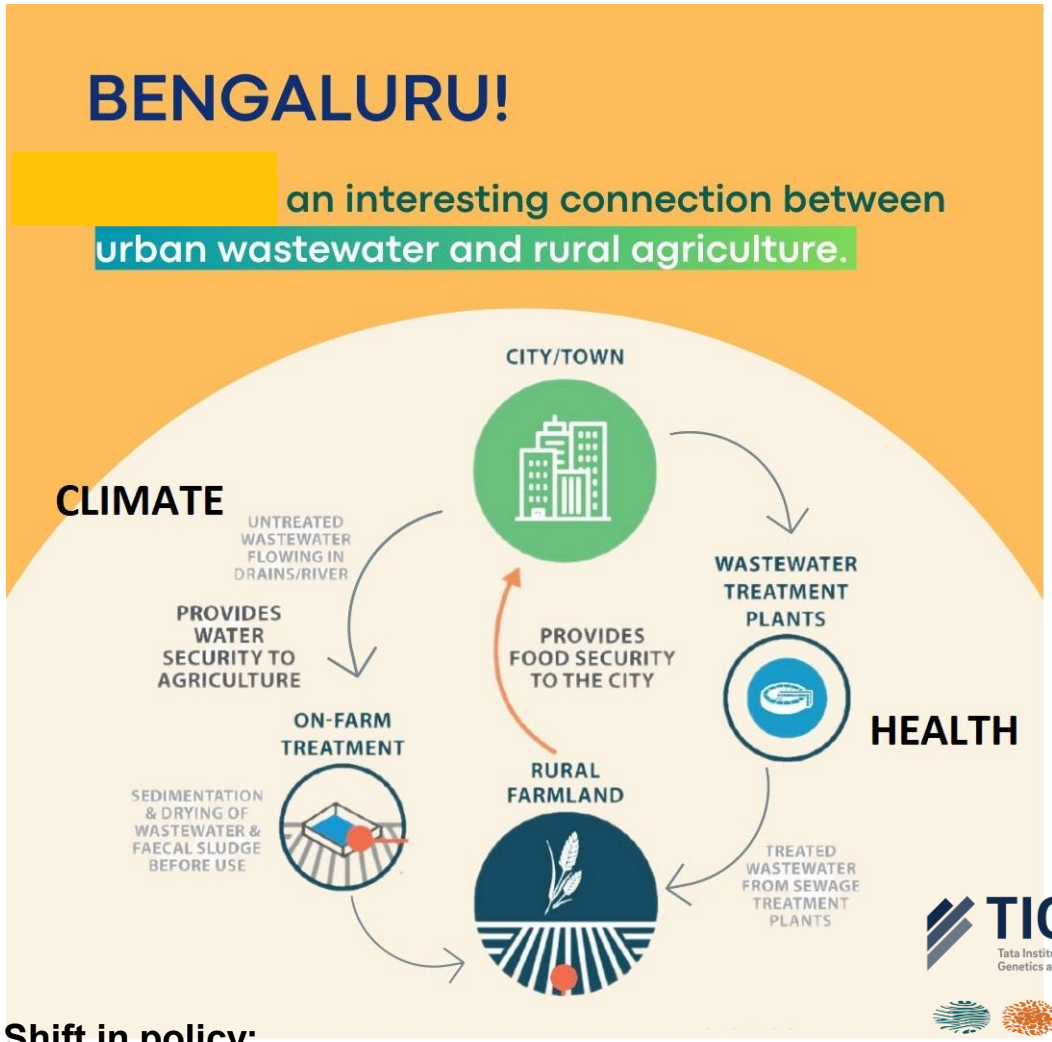
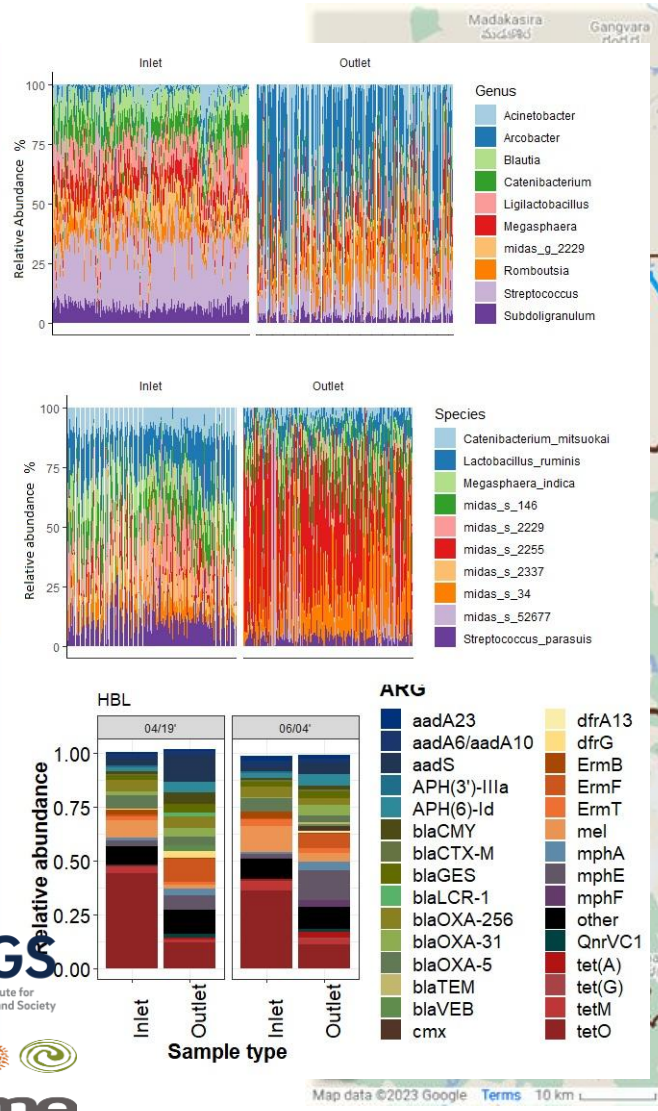
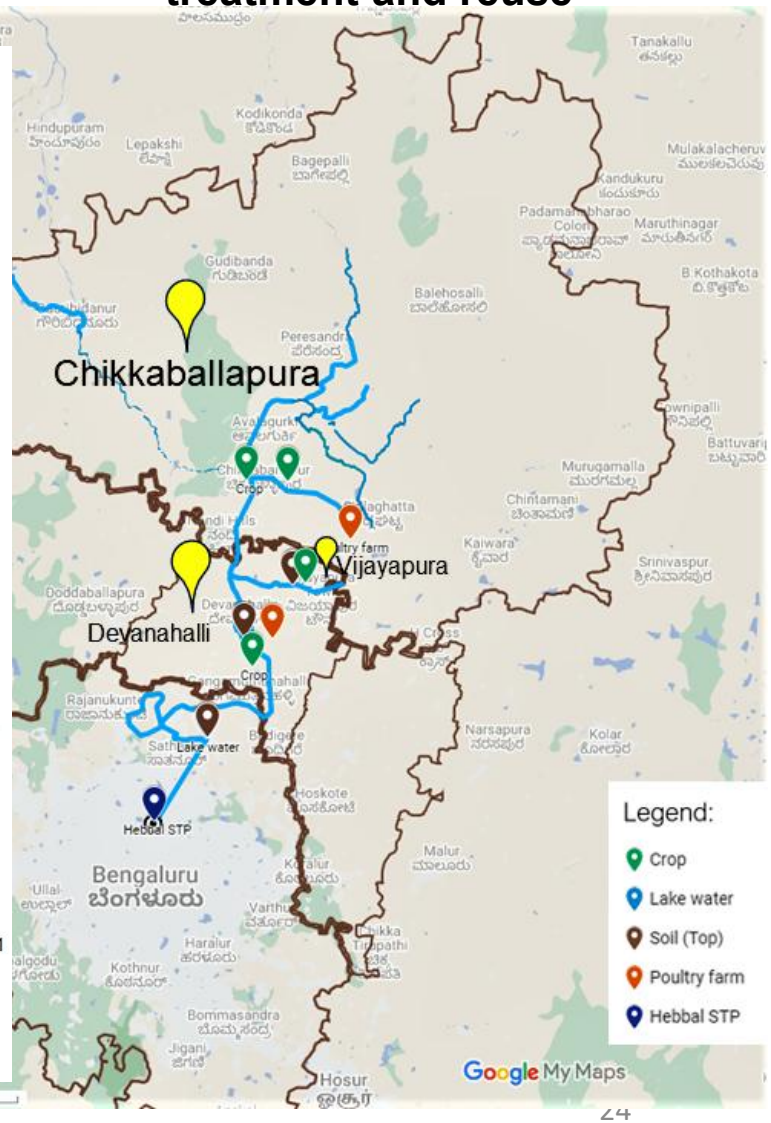
Name of the assay	Limit of detection	Type of patent	Technology transferred	Status/Challenges
Hepatitis A	10 viral copies/µl	Final Patent filed	Huwel Lifesciences	Completed
Hepatitis E	10 viral copies/µl	Final Patent filed	Huwel Lifesciences	Completed
Hepatitis B	10 viral copies/µl			Awaiting Industry partners response
Hepatitis C	10 viral copies/µl			Awaiting Industry partners response
DENCHIK(D1+D2+D3+D4+CHIKV)	10 viral copies/µl			Seek industry partners
DCZ (DENV+CHIKV+ZIKA)	10 viral copies/µl			Seek industry partners
TBEV+CCHFV+KFDV	10 viral copies/µl			Need help in Clinical validation and Seek industry partners
Scruptos (Leptospirosis+Scrub typhus)	10 bacterial copies/µl	Final Patent filed	Not Yet	Seek industry partners
Fever Panel	10 bacterial/viral copies/µl	Provisional Patent filed	Not Yet	Seek industry partners
Typhoid assay	10 bacterial copies/µl	In process	Assay with ICMR	Seek industry partners

Urban Water Footprint: Climate change, One Health, Circular economy

potential to address water scarcity through wastewater treatment and reuse



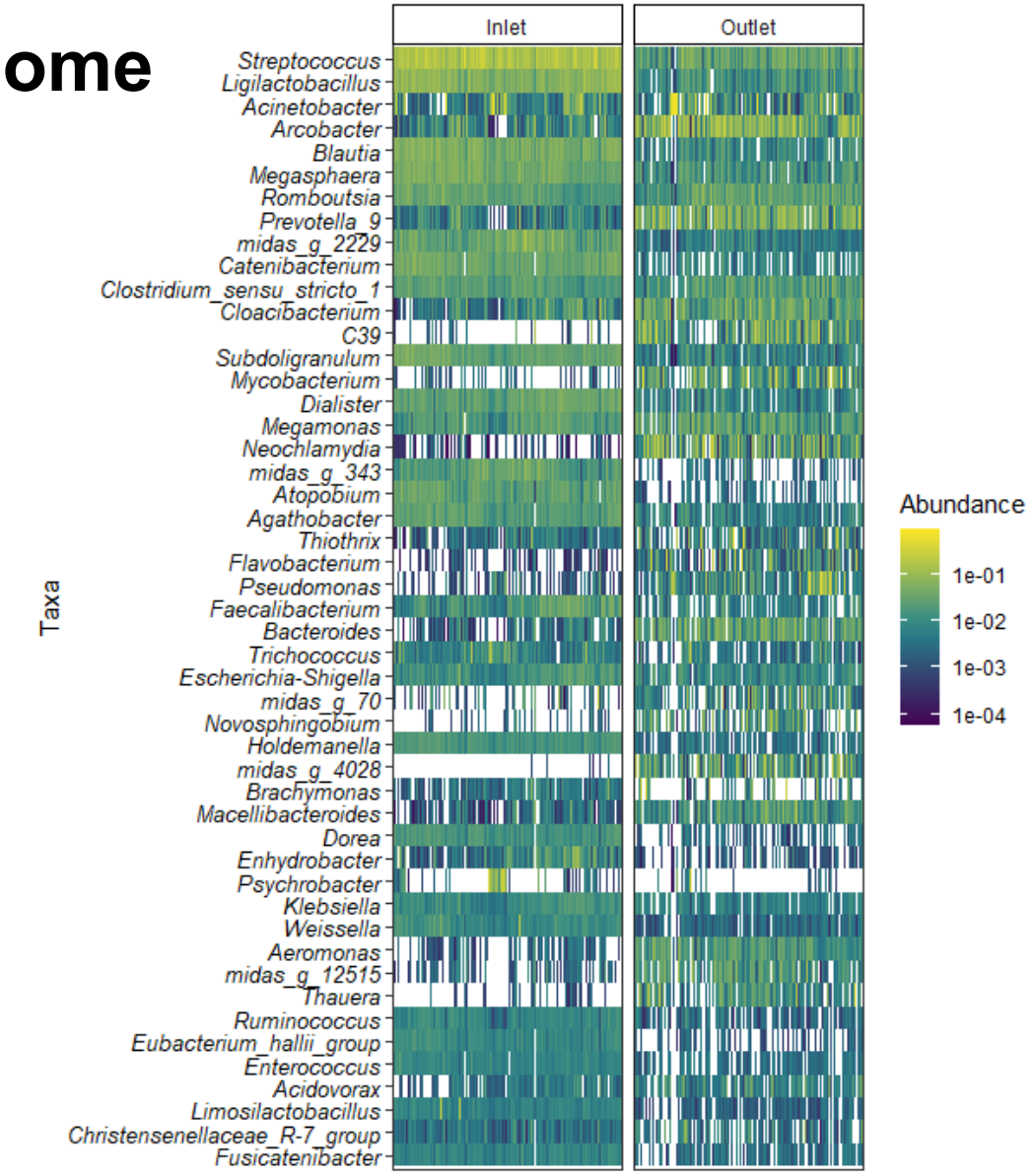




Shift in policy;
Change in efficiency of sewage treatment plants

How efficient are sewage treatment plants?

Sewage microbiome



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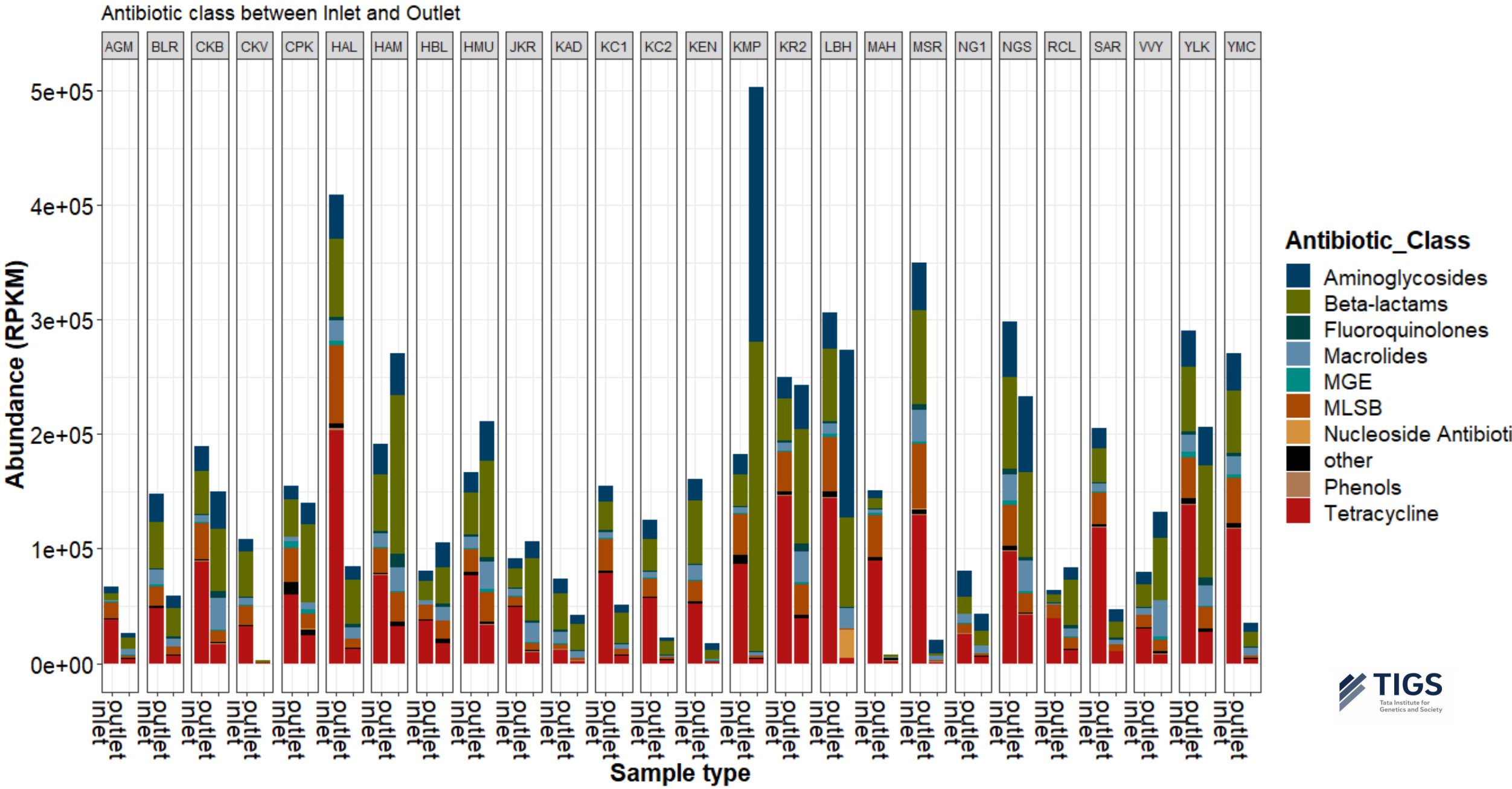
Antibiotic class



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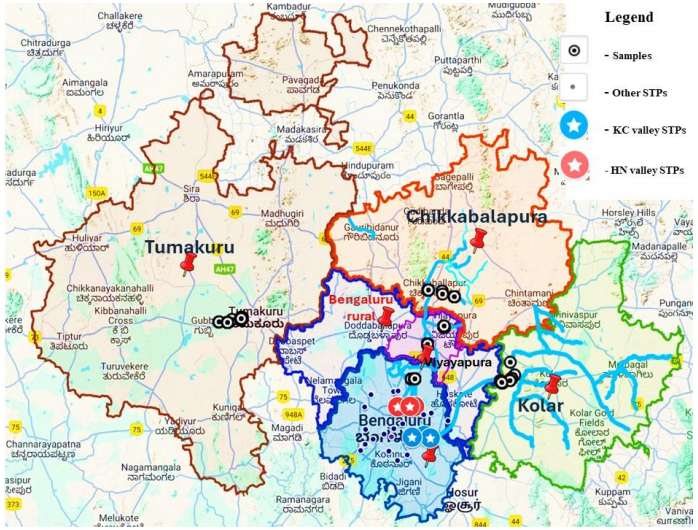
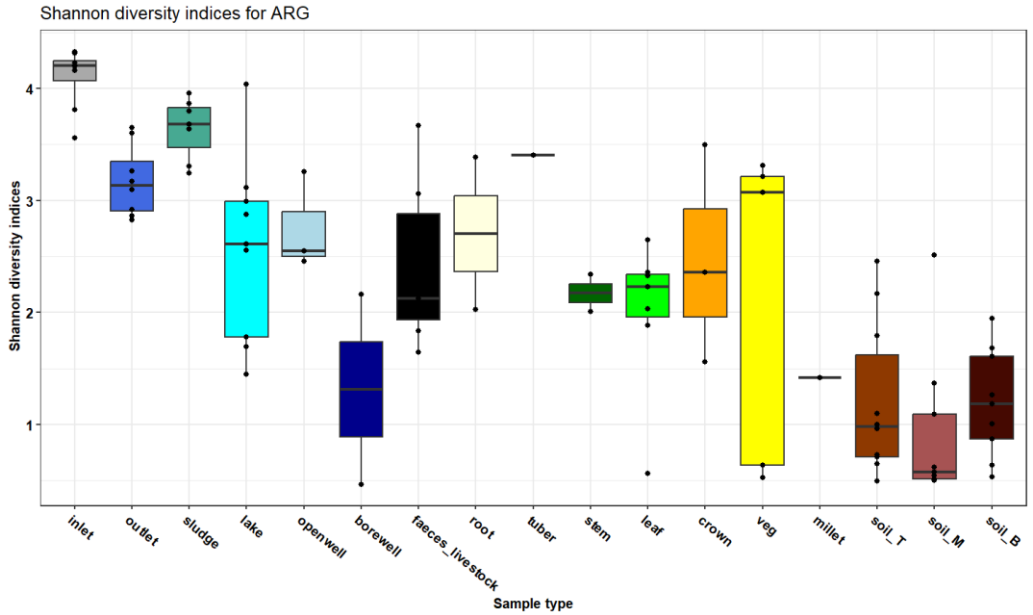
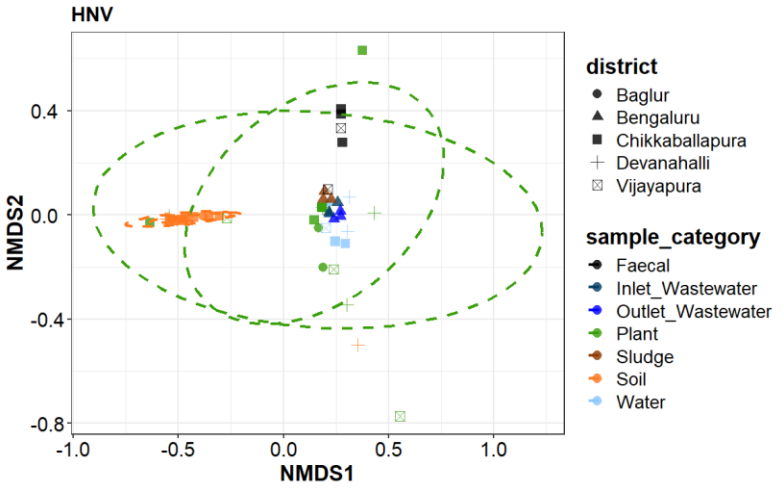
Urban Water Footprint: Climate change and One Health



Urban sewage to Rural sites:
Dilution in antimicrobial resistance genes (ARG)





Nataraj.. Farah Ishtiaq (2024) Metagenomic analysis reveals differential effects of sewage treatment on the microbiome and antibiotic resistome in Bengaluru, India. *Water Reuse*. https://doi.org/10.2166/wrd.2024.032

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Environmental Surveillance is an excellent tool to infer prevalence of pahthogens, AMR genes and antibiotics

1. Intricate mapping of sewershed areas: Identify hotspots/sites catchment areas
2. Integration with healthcare system for long-term monitoring of panpathogens: HPV, enteric viruses, AMR, bacterial, fungal etc.
3. Expansion of sites in peri-urban areas for AMR and other pathogens
4. Establishing sampling framework in different niches
5. Diagnostic protocols/kits at remote settings for quick identification of hotspots

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Strength of our work is in collaborations with interdisciplinary teams

- Theme Lead for One Health Bengaluru City Consortium (along with Dr. Uma Ramakrishnan, NCBS)
- Disease Surveillance thematic group of National Wildlife Health Policy
- Member of the Wastewater Surveillance Expert Working Group of the Asian Pathogen Genomics Initiative

Launch of One Health Cell Sub Committees

BBMP has constituted One Health Cell consisting of 5 committees:

Clinical Health
Public Health
Animal Health
Environmental Health
Digital Health

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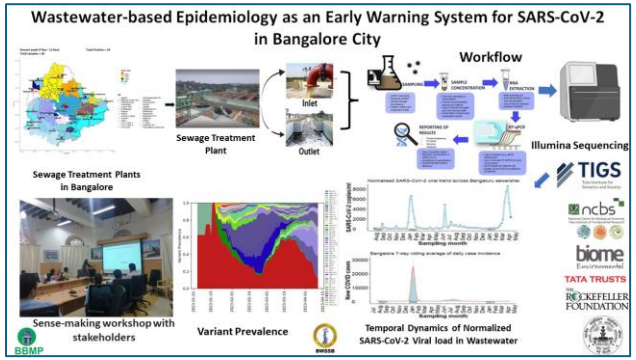
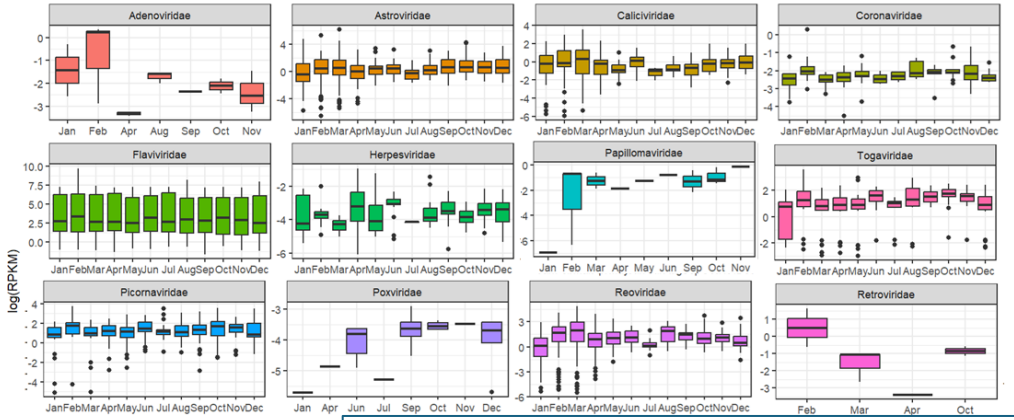
Integrating human and animal health with surveillance and disease ecology in a global urban center

Alliance for Pathogen Surveillance Innovations-India
data.ccmb.res.in

Phase 1: Wastewater surveillance as a complementary tool for monitoring SARS-COV-2 spread and evolution, emergence of new variants





Aug 2021

Aug 2022

Feb 2023

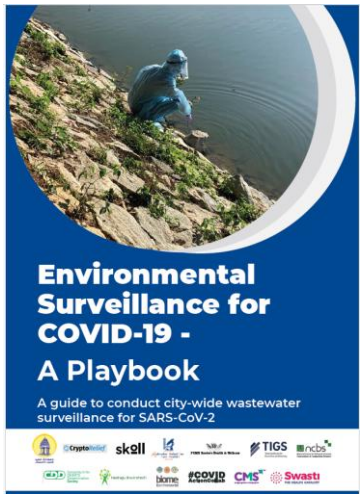
Jul 2023

Jul 2024

Hospital sampling for AMR and pathogen comparison with city sewage/ diagnostics

Dec 2024

Phase 2: Scaling up monitoring to other pathogens and capacity building



5th March 2023

Science group launches 'One Health' consortium

BENGALURU, DHNS: The Bengaluru Science and Technology (BeST) cluster announced the launch of its latest initiative — 'One Health Bengaluru City Consortium' — on March 3.

The aim of the consortium is to bring together experts from diverse fields to collaborate on building a comprehensive one-health framework for Bengaluru.

The BeST cluster, situated on the Indian Institute of Science (IISc) campus, is an initiative spearheaded by the Office of the Principal Scientific Advisor (OPSA) to the Union government.

Their latest project , One Health Bengaluru City Consortium, takes a holistic approach that recognises the interconnectedness of human, animal, and environmental health.

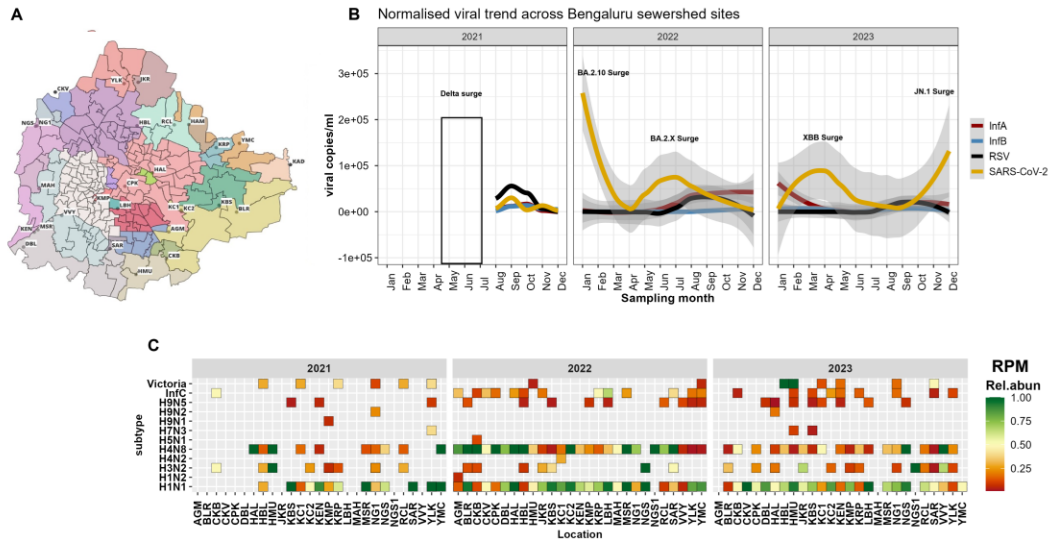
Dr Sindura Ganapathi, Visiting Fellow, One Health Mission, OPSA, said models for implementing 'One Health' in cities and districts have not been worked out.

Sewage surveillance

Sewage surveillance in Bengaluru should go beyond Covid to cover region-specific diseases, and the learnings should be used to develop models for other cities, he said.

BBMP Special Commissioner (Health) Dr Trilok Chandra said that the Palike has already established its One Health cell, a hi-tech laboratory, and a Metropolitan Monitoring Centre to capture major disease parameters.

Representatives from over 25 organisations, including researchers, industry experts, policymakers and NGOs, were present at the event.



Initiation of collaboration with other states-

Kerala (KCDC)
Tamil Nadu (TN OHCC)

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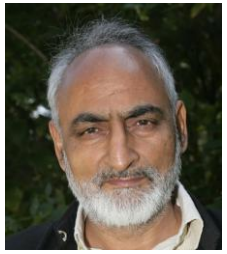
People behind this project

















Vivek C

Sebanti Tewary

Padmanaidu C Dammu

Partners

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biome Environmental

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National Centre for Biological Sciences
Tata Institute of Fundamental Research

BBMP

BWSSB

Indian Council of Medical Research

TATA TRUSTS

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Bill & Melinda Gates foundation