APSI Alliance for Pathogen Surveillance Innovations – India

Site selection guidelines for environmental surveillance of pathogens for public health action

Protocol Handbook 2024

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Introduction:

Disease surveillance is essential for a strong public health system. Surveillance is central to infectious disease epidemiology helping to understand the extent of disease burden and identify vulnerable and high-risk populations. Traditionally, disease surveillance has relied on detecting infected individuals within a population. However, direct estimates from such data can be biased due to underreporting and are often compounded by nonspecific febrile symptoms (e.g., fever, swollen lymph nodes, malaise), limited molecular diagnostics and asymptomatic infections [1-3].

Environmental Surveillance (ES) offers a powerful tool for monitoring several neglected and emerging infectious diseases. ES is characterized by the systematic collection of environmental media (e.g., water, wastewater, soil, air) from selected locations, analysis of nucleic acid in samples to detect, quantify and characterize infectious disease pathogens. While ES does not provide individual data, it is cost-effective and is particularly useful in low-resource settings where obtaining representative data on the infectious disease burden is especially challenging. Wastewater sampling has become an integral component of ES in more than 50 countries covering over 3000 sites for providing near-real time information on community exposure to COVID-19 [4].

The main purpose of ES is to analyze and collate the data for the purpose of informing decisions. Therefore, the selection of sites for ES depends on water-based systems where human sewage is present with the goal of improving public health. The selection of sampling sites can include convergent sewer networks that feed into treatment works, storm drains, septic tanks or rivers and lakes where untreated sewage is disposed into surface waters. Additionally, surveillance can be focused on high foot fall sites such as airports, flights, hospitals, schools, prisons by monitoring outflowing sewage at a building or compound level.

1. Types of Sites for Environmental Surveillance:

Selection of specific environmental sites should be based on the risk assessment, epidemiological situation and the expected role of ES in the broader context of disease surveillance. In addition, the frequency of sample collection is determined by the epidemiological question and pathogen in consideration (Table 1 from Ishtiaq *et al.* [5]).

Permanent sites:

Samples from permanent sites are expected to be routinely collected, and at minimum, on a weekly or bimonthly basis depending on the surveillance objectives.

Temporary sites:

Sites are selected to enhance surveillance in areas temporarily at high risk of pathogen circulation due to an outbreak or sudden influx of an at-risk population. The program should aim

to stop sample collection from these sites within a few months or years, once the outbreak is controlled. Some sites may be open only during specific times of the year ("seasonal"), in which case the expected sample months per year are site-specific.

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	Open drains & other satellite sites		Quantitative PCR	Digital Droplet PCR (ddPCR)
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, Chikumgunya, Zika		■	■	■		■	■	■	■		■			■		■			■		■	■		■	■
Norovirus							■		■			■	■			■			■			■		■	
Human Paplimoma Virus (HPV)							■		■				■				■		■			■		■	
Candida auris infection							■	■	■			■	■	■				■	■	■	■	■		■	
Antimicrobial resistance																									
Antimicrobial resistance genes				■	■	■	■	■	■				■					■	■			■			
Microbial diversity				■	■	■	■	■	■				■					■	■			■			
Fungal diversity				■	■	■	■	■	■												■	■			
Emerging contaminants																									
Antibiotics				■	■	■	■	■				■	■					■	■	■	■	■			
Antivirals				■	■	■	■	■				■	■					■	■	■	■	■			
Antimalarials				■	■	■	■	■				■	■					■	■	■	■	■			
Acetaminophen				■	■	■	■	■				■	■					■	■	■	■	■			
Caffine				■	■	■	■	■				■	■					■	■	■	■	■			
Disinfectants				■	■	■	■	■				■	■					■	■	■	■	■			
Heavy Metals			■		■		■	■					■	■				■	■	■	■	■			

Table 1 shows examples of pathogen and sampling frequency based on research questions

2. Selection of sampling sites

Once areas of epidemiological interest (e.g., surveillance of SARS-CoV-2, polio, hepatitis viruses) have been selected within a region, workshops and field visits must be conducted to identify feasible and cost-effective sewage collection sampling points.

Site selection assessment must consider following factors:

2.1 Size of catchment population

The number of people living in the catchment area of an ES site (i.e., those connected to a converging sewer network) affects the sensitivity of pathogen detection in a population. This sensitivity depends on the shedding rate.

For example, a catchment population of approximately 100,000 to 300,000 individuals is recommended as the optimal size for isolating poliovirus if it is circulating in the population. Since not all individual households may contribute fecal material to the wastewater flow or systems, it may be necessary to identify areas with larger overall populations. Systematic environmental sampling is estimated to detect one individual excreting wild poliovirus among 10,000 inhabitants. Similarly, the median viral load in wastewater for arboviruses ranges from 1E6 to 5E7 genome copies per person per day, which aligns with estimates for SARS-CoV-2 in wastewater. For 500 mL of wastewater, the sensitivity will range from a disease incidence of 1 in 50 to 1 in 2,500 people, implying that it could work well for smaller catchment areas. Positive detection requires a minimum of 20 infected persons in a catchment of 1,000 people and 200 infected persons in a catchment of 10,000 people [6].

2.2 Type of sewer systems

When identifying a sampling site, the surveillance programme should consult with both local sanitary engineers and epidemiological experts who can assist in evaluating sewer and wastewater systems in the area and provide information of the catchment populations. Below are the types of sewer systems that can be considered for sampling.

a) Converging sewer networks connected to household water drainage are optimal for systematic ES. The best location for sampling sites is the inlet closest to the treatment plant, where wastewater containing human faecal material from a larger population can be collected. For treatment facilities serving large metropolitan areas, the catchment area may be segmented by moving the sampling point upstream to inlets near pumping stations or major collector pipes to ensure a target large population (~100,000–300,000). A detailed epidemiological analysis of the population in each segment can guide the selection of sub-sites with representative groups at risk for disease transmission.

A detailed sewage network map and clearly defined micro sewersheds will help in the selection of sub-sites that can be scaled up during peak outbreak periods to identify disease hotspots and scaled down during lean periods (Fig.1).

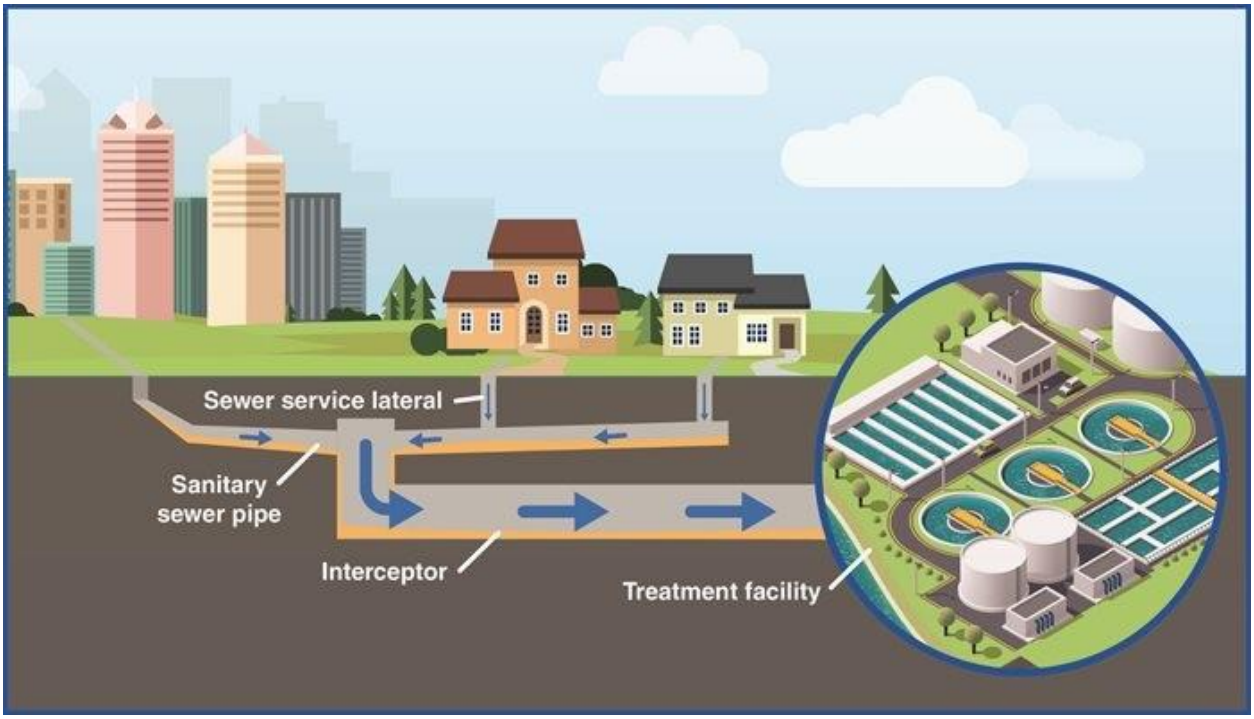


Fig. 1: Example for converging sewer network

b) Storm drains and open canals:

In low-resource settings, storm drains, or open canals are often the only available options. It is important to conduct a thorough exploration and mapping of the wastewater drainage in collaboration with local sewer engineers before selecting sampling points. Mapping sewer lines to detect blockages or obstructions will help exclude areas of the catchment population and identify potential sources of toxic waste in the wastewater.

In areas without detailed information on sewage network maps, global positioning system (GPS) coordinates along the wastewater paths will allow for the creation of "blue line maps" that can be modeled with specific computer software to estimate the catchment population for a specific sampling site (e.g., Uzell *et al*. [7] and Wang *et al*. [8]).

c) Open fields, latrines and septic tanks:

For epidemiological sampling, avoid areas where human waste is disposed of in latrines, septic tanks, or open fields without a convergent sewer system. Sampling in sewage treatment facilities where trucks dump raw sewage from septic pits and latrines is also not recommended, as the catchment population is likely small and difficult to estimate, and access usually depends on disposal fees rather than geographical location. Additionally, sampling from sites with insufficient wastewater flow may result in lower surveillance sensitivity due to undefined or uncontrolled factors, such as environmental inactivation of viruses.

Open fields can be considered for soil sampling for antimicrobial resistance genes and other public health parameters.

3. Accessibility:

It is also important to assess transportation logistics and accessibility, when selecting environmental surveillance sites, as collectors will need to walk and stand in public areas for approximately 30 minutes or longer to complete procedures. Because most sampling is recommended early in the day to coincide with peak toilet use, access to the site should be confirmed during the early morning hours. Areas that are intermittently inaccessible because of flooding or other seasonal considerations should only be considered for temporary environmental sites and only in specific situations, such as an outbreak.

A. Site selection for sewered communities: Following above guidelines, below is a brief description of sites considered for environmental surveillance of SARS-CoV-2, influenza, antimicrobial resistance across two cities with varying sewage networks

Pune:

a. Points considered for selection of environmental surveillance sites:

Pune city has 10 sewage treatment plants (STPs) spanning almost 65-70% population, and 1 National Chemical Laboratory (research institute)-NCL campus STP.

b. Approximate population near site:

Every STP site has an approximate population of 3-4 lakhs. NCL campus STP covers approximately 2000 residents including employees and students.

Bengaluru:

a. **Points considered for selection of site:**

Bengaluru has a centralized sewage network serving 70% of the population. The city has 28 sewage treatment plants that have been monitored for SARS-CoV-2 since 2021 (Fig. 2).

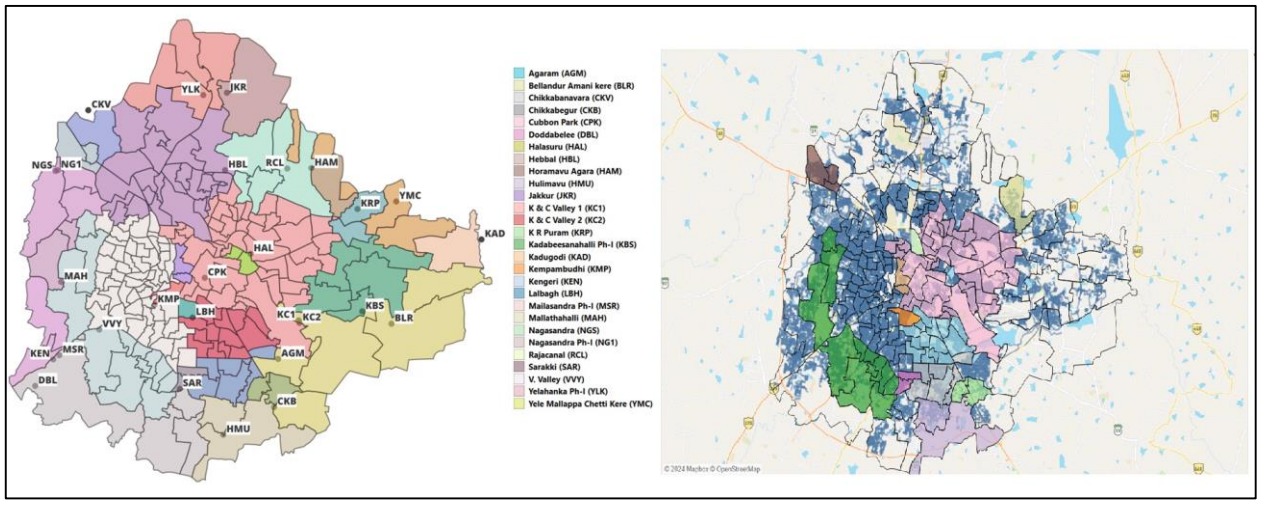


Fig. 2: Map of Bengaluru city with a sewage network serving 70% of the population.

b. **Approximate population near site:**

These STPs covered different catchment area sizes: eight small sizes (serving 10,000-60,000 population), nine medium sizes (serving 100,000 – 350,000 population), and nine large sizes (serving 400,000 to 2,480,000 population) STPs

B. Site selection for non-sewered communities:

A few examples of site selection of non-sewage treatment plant wastewater bodies such as open drains, rivers and lakes are cited below (location-wise):

<u>Delhi/NCR/Sonipat:</u>

Points considered for selection of sites:

a. Geographical representation of the area being covered: Two cities were covered for sampling: a smaller peri-urban area (Sonipat) and a large urban area (Delhi). For Sonipat, at least three geographical locations on existing drains, including the immediate point where the effluent enters the drain was chosen. For Delhi, sampling sites covering a broad representation of the North and South parts of the city were chosen.

b. Population density: For the Delhi-NCR region open drains which were reported to have the highest volumes of water, and juxtaposed with areas of high population density were selected. Based on population density, drains/sites were selected.

c. Infrastructure and accessibility: Selected sites based on practical constraints such as accessibility in existing sewer networks were considered.

d. Logistic and regulatory compliance: Sites were mapped where permission for regulatory compliance could be accessed regularly

e. Open sewers connecting directly from residential houses: Within a drain, some areas were selected for sampling, where the sewage does not go to a STP but enters the drain directly from pipes outside the house.

f. Special sites: Finally, some sites were selected with the intent to monitor specific locations. For example, a site immediately outside a hospital was selected where clinical samples from patients were being obtained to correlate wastewater data with clinical data on presence of specific pathogens. Similarly, part of the peri-urban area is housed in a heavily industrial zone. One such catchment area site within the drain surrounding a commercial area was selected-this area had heavy human contact (people bathing, washing, etc.) where the industrial efflux was also being released.

g. Approximate population near site: Based on geo-locations and pincode based assessment from the 2011 census:

Sonipat, Haryana - 50,128 (4 Areas, 10-11 Sites)

Delhi - 3,63,000 (13 Areas, 14-15 Sites)

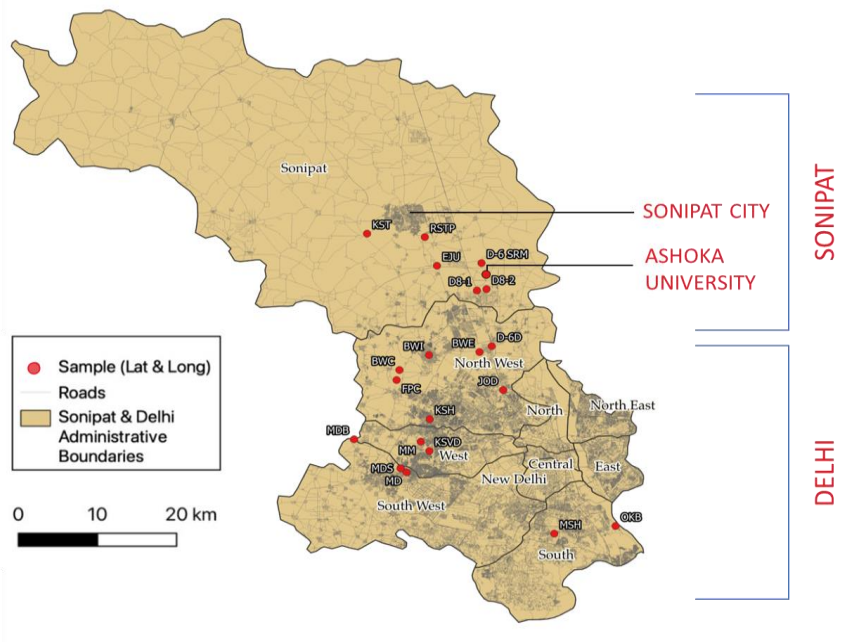


Fig. 3. Map of Sonipat/Delhi regions depicting wastewater sampling sites (red dots).

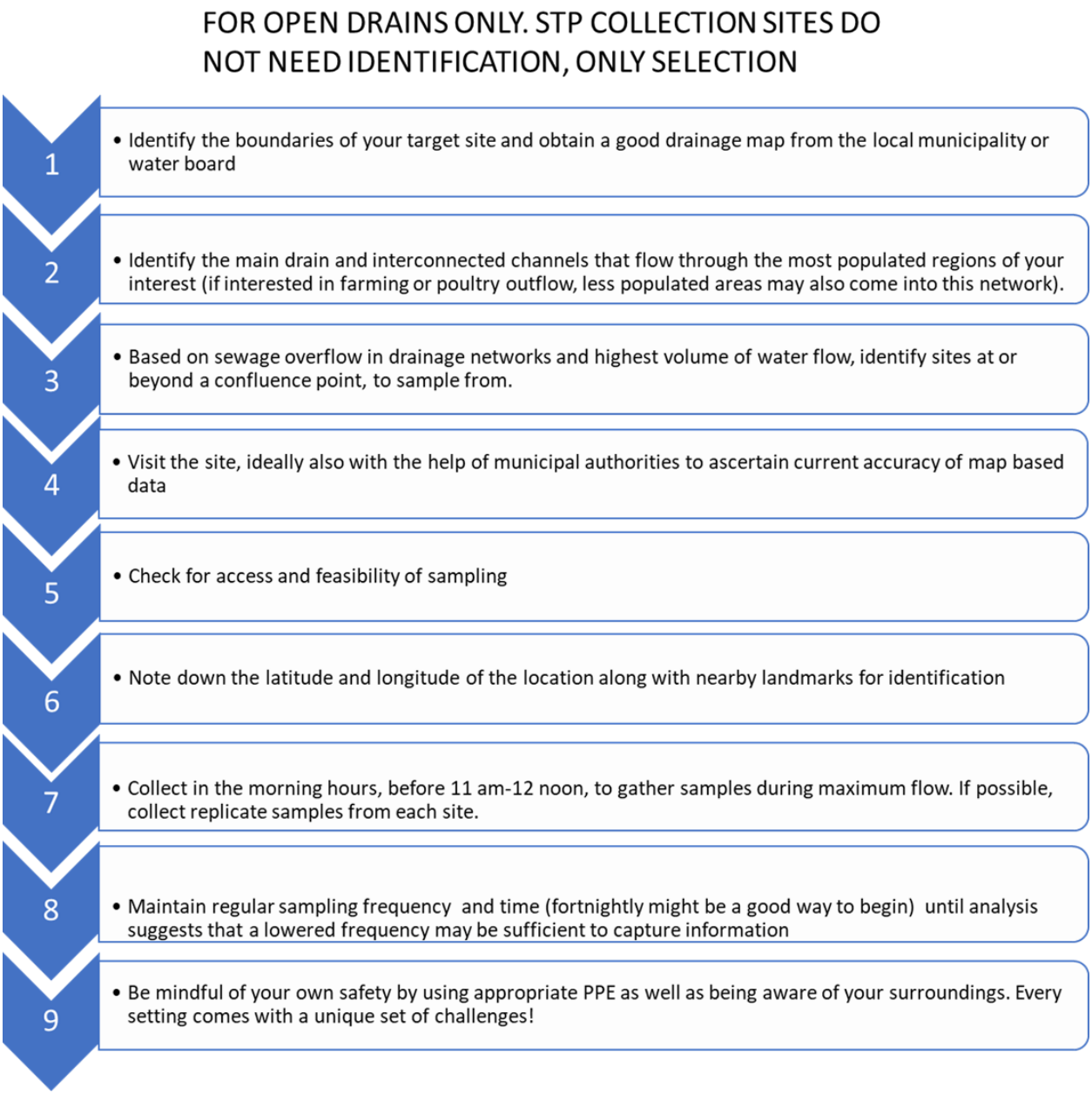


Fig. 4: Criteria for choosing wastewater sampling sites in Sonipat/Delhi regions.

Hyderabad:

a. Points considered for selection of site:

- Hyderabad has a population of around 10 million, as of 2023. The city generates around 6246 million litres sewage daily, of which around 2922 million litres is treated through the sewage treatment plants in the city and the remaining volume finds the way in the open drainage system. Thus around 53% sewage goes into drains in Hyderabad.

- The population density is very high around Musi river in Hyderabad. Musi river receives sewage influx from the densely populated regions in the city. Many of the sampling points are from the wards around Musi river. About 500 million litres of sewage joins in the river daily.

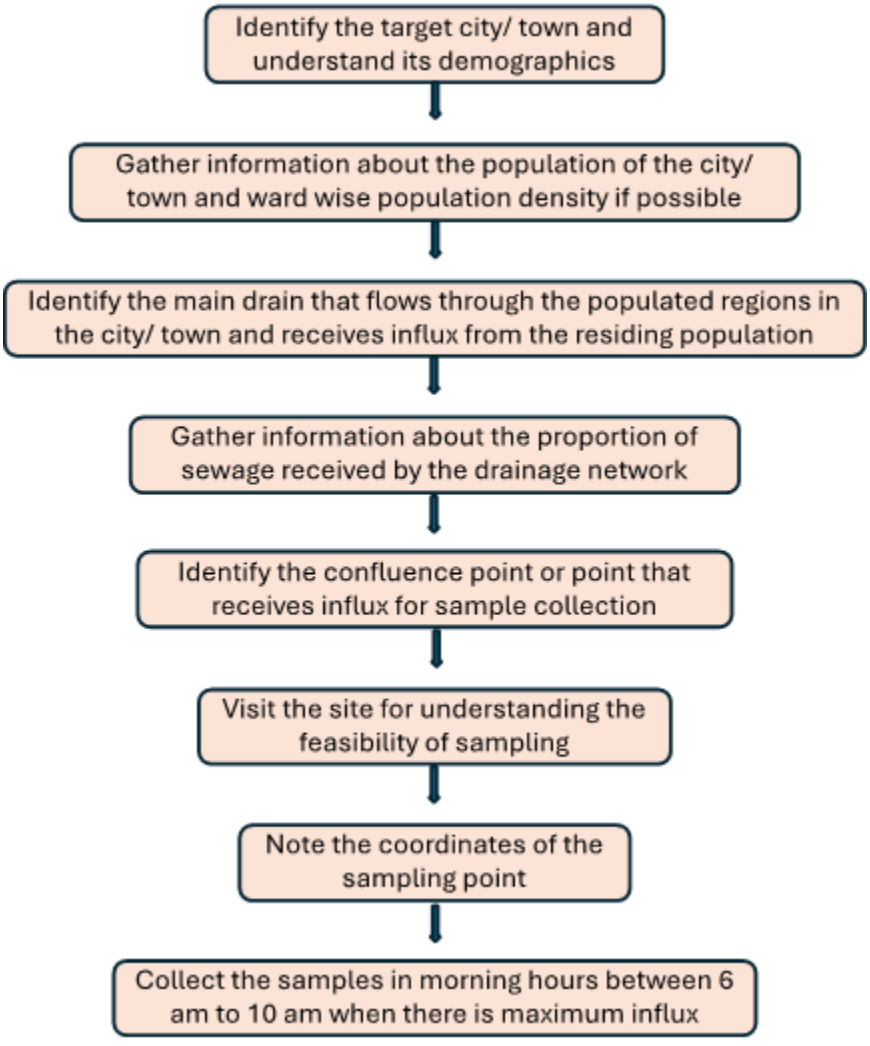


Fig. 5: Criteria for choosing open drain wastewater sampling sites in Hyderabad

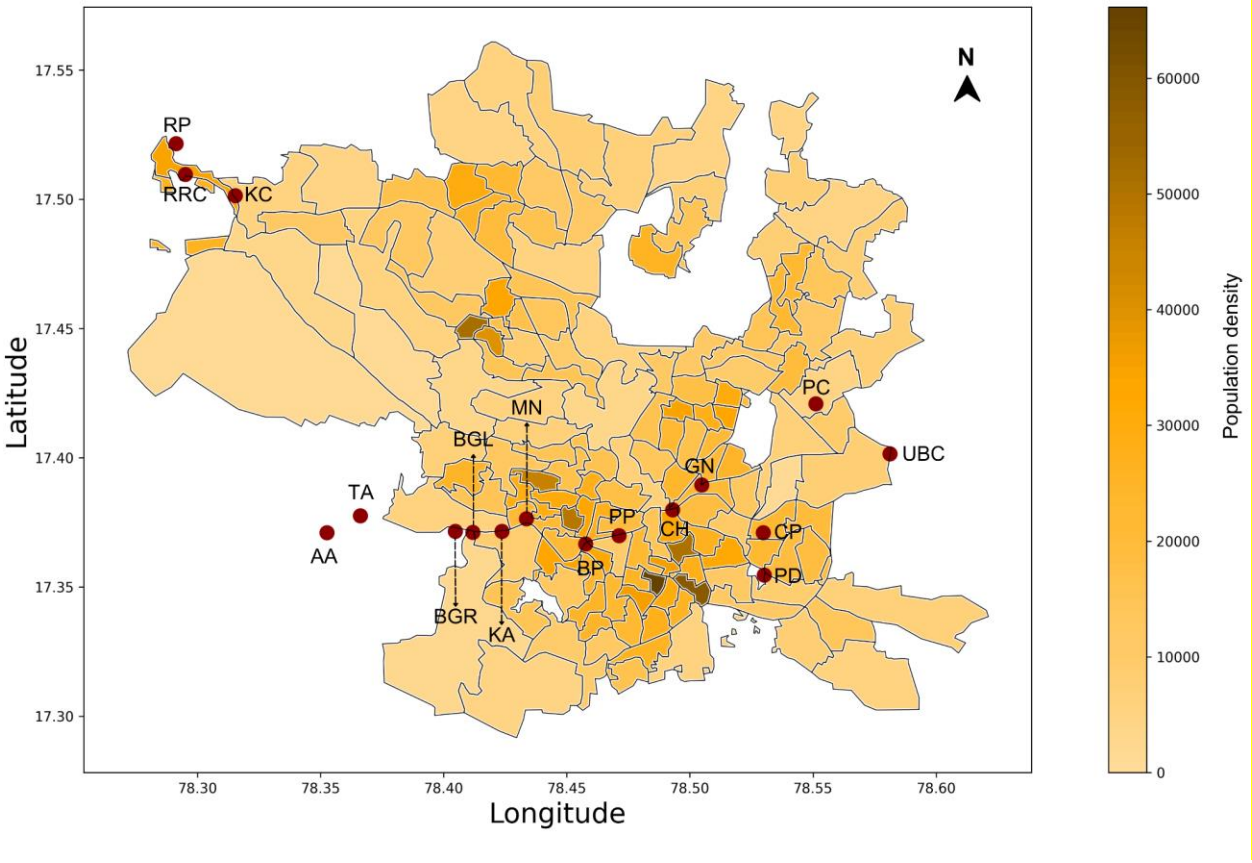


Fig. 6: Map of Hyderabad city depicting wastewater sampling sites (red dots) along with their population density.

Shillong:

a. Points considered for selection of site:

When choosing the sites for wastewater sampling, the following are the inclusion criteria:

a. Residential coverage of the area selected, with a minimum population of 5000 within the area represented.
b. Feasibility with field human resources such as ease of collection etc.

The exclusion criteria for wastewater sampling are:

a. Areas near hospitals and heavy industries.
b. Any sites not practical for field sampling, such as the area around a manhole that does not offer enough space or safety for field personnel.

In Shillong there are no STPs, hence, by applying the above inclusion and exclusion criteria, the following points have been taken into consideration:

1. The Shillong agglomerate is encircled by two rivers, Umshyrpi and Wahumkhrah. The selected sites are open drains/streams along the river bed which eventually drain to either of these rivers. Therefore, these sites are feasible for wastewater collection as they are natural drainage and catchment areas due to the natural geographical location and the typography of the region.

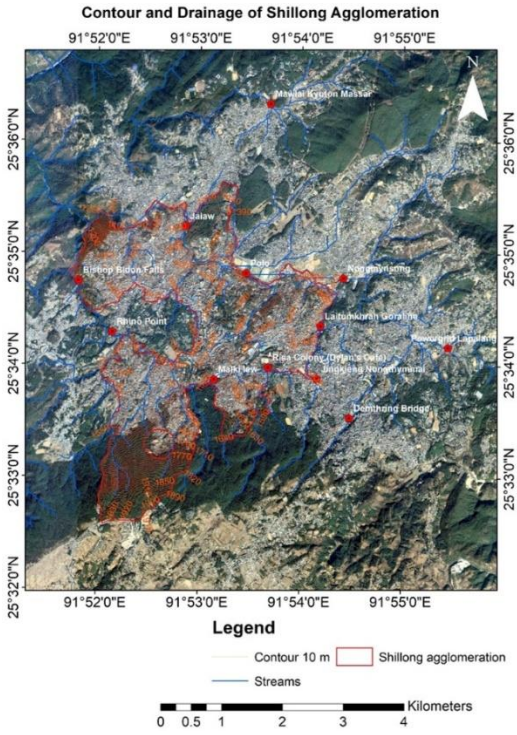
2. The sites selected are representative of the overall population of the urban agglomeration of the city.

3. These sites provide good information since they are near to or drain from the major residential areas of the city.

4. Ease of accessibility for collection.

    b. Approximate population near site:

Number of sites selected from Shillong: 12



Sl. No.	River	Location	Latitude	Longitude	Population (Census 2011) *
1	Umshyrpi River	Malki Iew	25.56458245	91.88586110	22,877
2	Umshyrpi River	Risa Colony (Dylan's Café)	25.56638547	91.89470513	12,549
3	Umshyrpi River	Jingkieng Nongthymmai	25.56473947	91.90269635	38,004
4	Umshyrpi River	Rhino Point	25.57158367	91.86918562	34,993
5	Umshyrpi River	Bishop Bidon Falls	25.57919352	91.86358144	36,559
6	Wahumkhrah River	Powergrid Lapalang	25.56945198	91.92415148	21,524
7	Wahumkhrah River	Laitumkhrah Goraline	25.57257399	91.90327584	22,993
8	Wahumkhrah River	Demthring Bridge	25.55892544	91.90806633	29,194
9	Wahumkhrah River	Nongmynsong	25.57973223	91.90695513	42,236
10	Wahumkhrah River	Polo	25.58037958	91.89101892	23,674
11	Wahumkhrah River	Jaiaw	25.58731552	91.88108144	26,216
12	Wahumkhrah River	Mawlai Kynton Massar	25.60561141	91.89485924	55,012

Fig. 7. Map of Shillong depicting wastewater sampling sites (red dots) along with their population density.

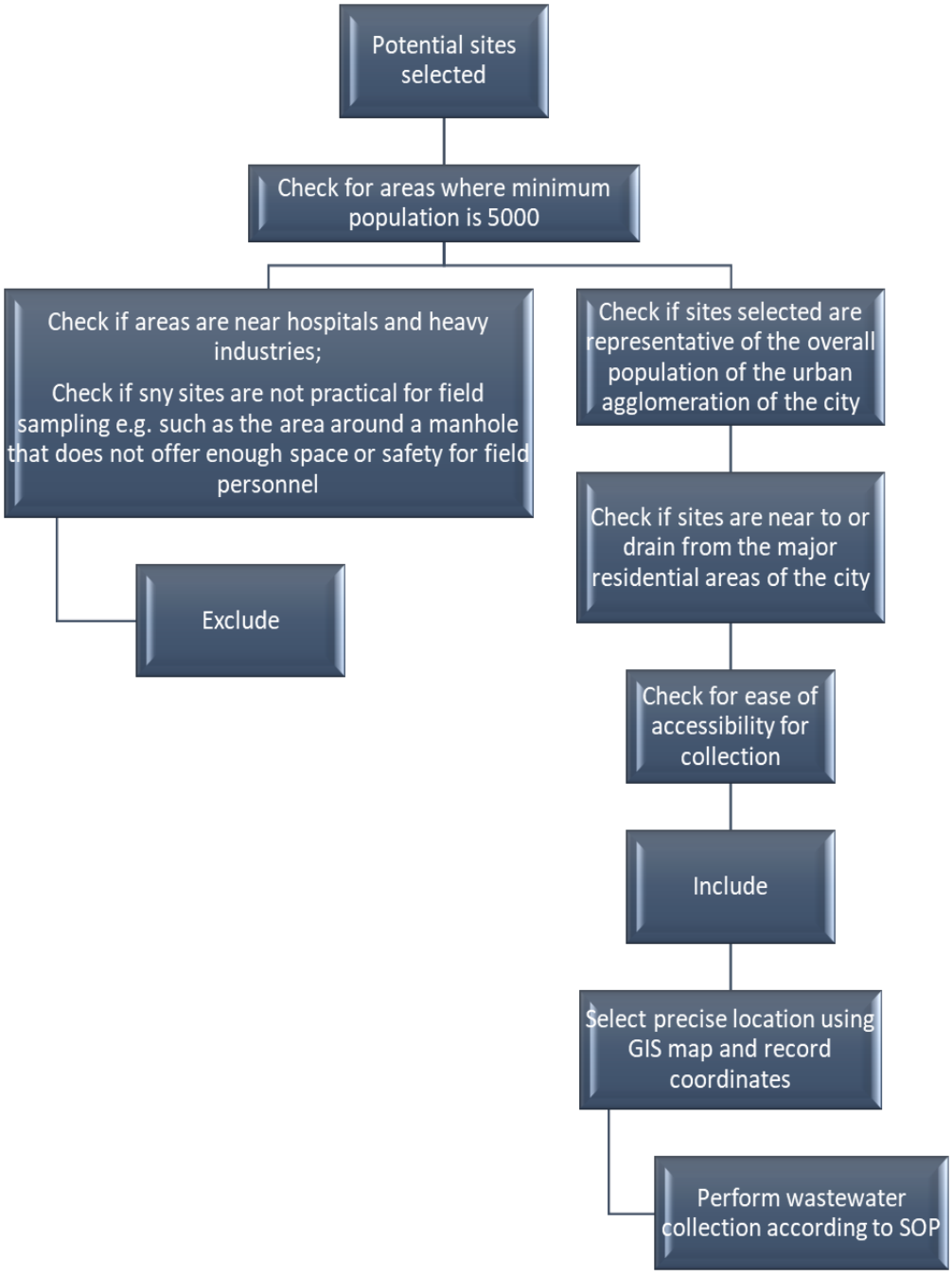


Fig. 8: Flowchart showing procedure to select wastewater sampling sites in Shillong.

References:

1. Diamond, M.B. *et al*. (2023) Wastewater surveillance facilitates climate change –resilient pathogen monitoring. *Sci.Transl. Med.* 15, eadi7831.

2. Ishtiaq F. (2023) Wastewater-based surveillance of vector-borne pathogens. *Trends in Parasitology*, 40: 93-95.

3. National Academies of Sciences, Engineering, and Medicine (2023) Wastewater-based Disease Surveillance for Public Health Action. The National Academies Press https://doi.org/10.17226/26767

4. COVIDPoops19. Summary of global SARS-CoV-2 wastewater monitoring efforts by UC Merced researchers. Univ Calif Merced;2022. URL596 https://ucmerced.maps.arcgis.com/apps/dashboards/c778145ea5bb4daeb58d31afee389082.

5. Ishtiaq, F et al. Defining a research agenda for wastewater and environmental surveillance of pathogens in low resource setting-an example from urban and semi-urban Bengaluru, Karnataka (manuscript)

6. Lee WL, Gu X, Armas F, Leifels M, et al. (2022). Monitoring Human Arboviral Diseases through Wastewater Surveillance: Challenges, Progress and Future Opportunities. *Water Res.* 2022, *223*, 118904.

7. Uzzell CB *et al*. (2023) Environmental surveillance for *Salmonella* Typhi as a tool to estimate the incidence of typhoid fever in low-income populations. *Wellcome Open Res.* 8, 9.

8. Wang *et al*. (2023) When case reporting becomes untenable: Can sewer networks tell us where COVID-19 transmission occurs? Water Research, 229:119516.

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