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Wastewater-based epidemiology in India: current status, prospects, and recommendations

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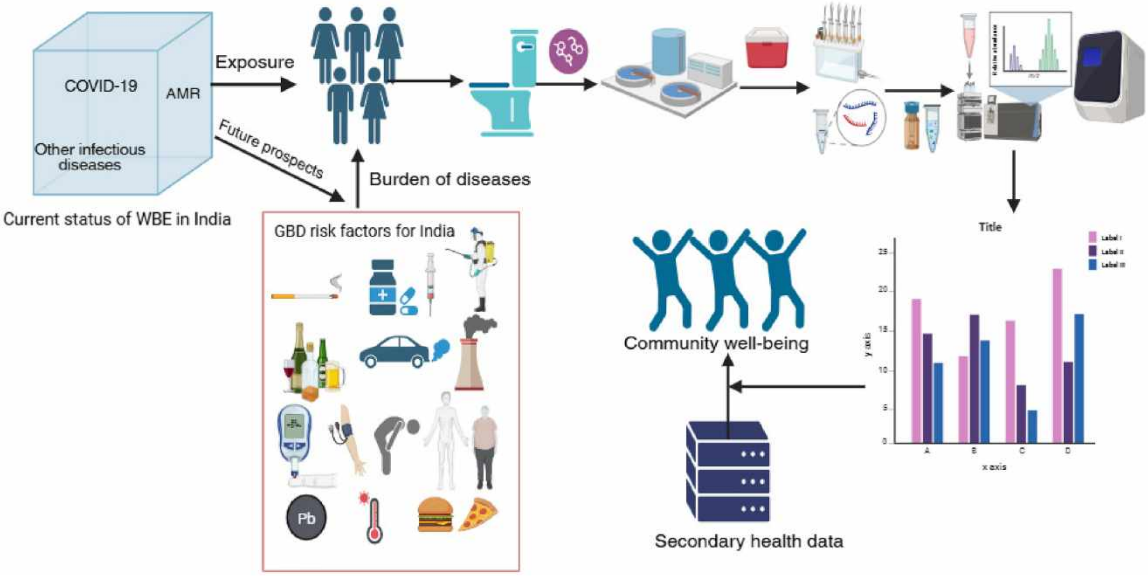
ABSTRACT

The dual burden of communicable and non-communicable diseases in India is putting a significant strain on the country's healthcare system. Conventional public health assessment tools alone cannot tackle multifaceted public health crises in India. There is a need for novel tools to monitor public health in near real-time, complementing existing health assessments. Wastewater-based epidemiology (WBE) is a technique that can provide essential information on the population's health and behavior within a given catchment boundary in near real-time by detecting urinary and fecal markers in municipal wastewater. Triggered by the COVID-19 pandemic, WBE attracted the attention of the scientific community in India to monitor the progression of COVID-19 disease in the population. WBE studies in India have mainly focused on monitoring infectious diseases and antimicrobial resistance. The existing network of sanitation infrastructure across India indicates a promising future for WBE in the country. This systematic review summarizes the work done so far in the field of WBE in India, discusses the challenges, and emphasizes the need to expand its horizon to unlock its full potential in the rapid identification of emerging health threats that contribute to the public health burden. A roadmap has been proposed for the nationwide implementation of WBE.

Key words: drug use, one health, public health, wastewater surveillance, zoonotic diseases

GRAPHICAL ABSTRACT



1. INTRODUCTION

The recent global burden of disease (GBD) study spotlighted the epidemiological transition in India in the last 20 years (IHME 2024). Disability-adjusted life years (DALYs) per 100,000 people and the number of deaths in India due to communicable, maternal, neonatal, and nutritional (CMNN) diseases have shown a downward trend from 1990 to 2021, whereas the non-communicable diseases (NCDs) have risen (IHME 2024). DALYs estimate the total number of years lost due to specific causes and risk factors. In simpler terms, one DALY equates to one lost year of healthy life (IHME 2024). Although India has made substantial progress in managing CMNN in the last 20 years, the country is still facing the dual burden of diseases due to CMNN and NCDs. The COVID-19 morbidity and mortality in India also showed a positive correlation with NCD risk factors, indicating a lethal link between communicable diseases and NCDs (Shrivastav *et al.* 2023). Triggered by the COVID-19 pandemic, India holds the unenviable 66th position among 195 countries on the Global Health Security Index (GHI) 2021, a measure of health security and related capabilities, including pandemic and epidemic preparedness (Nuclear Threat Initiative 2021).

Although indispensable, conventional public health assessment tools, including surveys, population censuses, vital records, clinical testing, hospitalization data, and health insurance claims, are insufficient to manage public health efficiently, as these are time-consuming, expensive, invasive, and may be prone to bias (Kumar *et al.* 2022a). Clinical detection methods for any disease focus on individuals, thereby rendering a fast community diagnosis cumbersome. A recent report published by the National Institution for Transforming India (NITI Aayog) also emphasized strengthening public health surveillance by 2030 (NITI Aayog 2022).

To address public health challenges, India needs a surveillance tool capable of rapidly identifying and tracking communicable diseases and NCDs and their risk factors in near real-time, ensuring more efficient pandemic preparedness, establishing robust early warning systems for diseases, and improving public health.

Wastewater-based epidemiology (WBE) offers numerous opportunities to address current public health challenges in India. It can help measure the health of communities residing within a catchment boundary on a near real-time scale by detecting water-borne human-excreted markers in wastewater. WBE was initially implemented to monitor illicit drug consumption in Western communities; however, its potential applications rapidly expanded. WBE has been globally employed to estimate substance abuse (Zuccato *et al.* 2008), pharmaceutical consumption, exposure to chemicals (Choi *et al.* 2018), antimicrobial resistance (AMR), and infectious disease prevalence (Medema *et al.* 2020) at the community level. WBE started gaining popularity in the pre-COVID-19 era, as evidenced by a significant growth in the number of publications in this field (Choi *et al.* 2018). However, the COVID-19 pandemic emphasized the demand for WBE, mainly in the space of pathogen detection and surveillance, across the globe, leading to its current use in at least 55 countries, covering about 300 million people (Adhikari & Halden 2022).

In the pre-COVID-19 era, sewage surveillance played a pivotal role in India in assessing the efficacy of the polio eradication program, which led to the official celebration of victory over polio in 2014 (Garg *et al.* 2018). The public health crisis sparked by the COVID-19 pandemic triggered the Indian scientific community to resume extensive sewage investigations to track the SARS-CoV-2 virus and inform authorities of upcoming surges in community-level infections (Kumar *et al.* 2020). Looking at the potential of WBE in tracking COVID-19, the Indian SARS-CoV-2 Genomics Consortium (INSACOG), a joint initiative of a group of ministerial bodies, directed all states in 2021 to monitor sewage to understand the type of genomic variants of the SARS-CoV-2 virus (Tomar & Khairnar 2023). Other remarkable WBE-related progress in India included the establishment of India's first city-wide wastewater surveillance system for the SARS-CoV-2 virus by the Bengaluru-based Precision Health platform in 2021 and the inclusion of sewage surveillance at selected sentinel sites under the umbrella of the proposed National One Health Mission (Business Standard, 2022; PSA 2024).

To envision the prospects of WBE in India, it is imperative to understand what has already been accomplished in this field and what should be the future goals, given the nationwide public health priorities and sanitation infrastructure. To the best of our knowledge, no prior study has compiled and reviewed the existing WBE literature in India and elucidated its prospects. This study presents the first systematic review of WBE in India, examining its current status and detailing its opportunities within the Indian context. This review also provides recommendations for successfully establishing the WBE protocol in India. The specific objectives of this study include (i) to conduct a systematic review of the literature following the PRISMA guidelines, (ii) to evaluate the utility of WBE as a public health surveillance tool in the context of India's current wastewater infrastructure and public health burden, and (iii) to provide evidence-based recommendations informing national policy design.

2. MATERIALS AND METHODS

The literature search was performed following the PRISMA 2020 statement (PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page *et al.* 2021). PubMed, Scopus, and Web of Science databases were searched for publications until 1st March 2025 without any filters applied for the year of publication. The Medical Subject Headings (MeSH) terms that were used include wastewater, waste water, sewage, faeces/feces, urine, surveillance, epidemiology, pandemics, disease, diseases, infection, disease outbreaks, public health, health care, environmental monitoring, virus, bacteria, COVID-19/SARS-CoV-2, poliovirus, influenza, hepatitis, enteric pathogens, illicit drugs, substance abuse, pharmaceuticals, antibiotics, AMR, PPCPs, and India. The search was performed using the Boolean operators 'AND' and 'OR'. The operator 'NOT' was used to exclude reviews and systematic reviews, literature reviews, and meta-analyses. Wherever applicable, the operators '' and () were used. The search results were compared based on the title and author names. The language was restricted to English. The full search strings used for each database are provided in Table 1. Duplicates were removed from the compiled search results using the delete duplicates function in Excel. This was followed by screening the titles to select the potential articles. By manually screening the titles and abstracts of the articles, the relevant articles were identified. Two independent reviewers (S.J. and A.D.) conducted the title and abstract screening as well as full-text assessment. Any discrepancies were resolved through discussion and consensus. This step was followed to minimize bias and maintain methodological rigor. This study weighted all compiled studies uniformly, which could introduce the risk of bias. This study acknowledges that heterogeneity and lack of quality grading may limit the strength of inference.

Publications identified were then screened for their full text according to eligibility criteria drawn out with the PICO tool. The PICO (Methley *et al.* 2014; Shah *et al.* 2022) terms used in this paper for selecting the articles are defined in Table 2.

3. RESULTS AND DISCUSSION

3.1. Status of WBE in India based on the systematic review

The following subsections provide an overview of the key findings from the systematic review conducted in this study.

3.1.1. Overview and evolution of WBE studies in India

A total of 1,025 studies were identified from the database search. The literature search identified records from PubMed (n = 351), Scopus (n = 347), and Web of Science (n = 327). Out of which, 397 duplicate studies were removed. 628 articles were further screened for the relevant title and abstract. Only 94 articles were fully assessed for their eligibility to be included in

Table 1 | Full search strings used for each database

Databases	Search strings
PubMed	((‘wastewater’ [MeSH Terms] OR ‘waste water’ OR ‘sewage’ OR ‘urine’ OR ‘faeces’ OR ‘feces’) AND (‘surveillance’ OR ‘epidemiology’ OR ‘pandemics’ OR ‘infection’ OR ‘disease outbreaks’ OR ‘public health’ OR ‘health care’ OR ‘environmental monitoring’) AND (‘virus’ OR ‘bacteria’ OR ‘COVID-19’ OR ‘SARS-CoV-2’ OR ‘polio’ OR ‘influenza’ OR ‘hepatitis’ OR ‘enteric pathogens’ OR ‘antimicrobial resistance’ OR ‘AMR’ OR ‘pharmaceuticals’ OR ‘antibiotics’ OR ‘PPCPs’ OR ‘substance abuse’ OR ‘illicit drugs’) AND (‘India’)) NOT (‘review’ [Publication Type] OR ‘systematic review’ OR ‘meta-analysis’ OR ‘literature review’)
Scopus	TITLE-ABS-KEY ((‘wastewater’ OR ‘waste water’ OR ‘sewage’ OR ‘urine’ OR ‘faeces’ OR ‘feces’) AND (‘surveillance’ OR ‘epidemiology’ OR ‘pandemics’ OR ‘infection’ OR ‘disease outbreaks’ OR ‘public health’ OR ‘health care’ OR ‘environmental monitoring’) AND (‘virus’ OR ‘bacteria’ OR ‘COVID-19’ OR ‘SARS-CoV-2’ OR ‘polio’ OR ‘influenza’ OR ‘hepatitis’ OR ‘enteric pathogens’ OR ‘antimicrobial resistance’ OR ‘AMR’ OR ‘pharmaceuticals’ OR ‘antibiotics’ OR ‘PPCPs’ OR ‘substance abuse’ OR ‘illicit drugs’) AND (‘India’) AND NOT (DOCTYPE (re) OR DOCTYPE (‘ar’ AND TITLE (‘review’)))
Web of Science	TS = ((‘wastewater’ OR ‘waste water’ OR ‘sewage’ OR ‘urine’ OR ‘faeces’ OR ‘feces’) AND (‘surveillance’ OR ‘epidemiology’ OR ‘pandemics’ OR ‘infection’ OR ‘disease outbreaks’ OR ‘public health’ OR ‘health care’ OR ‘environmental monitoring’) AND (‘virus’ OR ‘bacteria’ OR ‘COVID-19’ OR ‘SARS-CoV-2’ OR ‘polio’ OR ‘influenza’ OR ‘hepatitis’ OR ‘enteric pathogens’ OR ‘antimicrobial resistance’ OR ‘AMR’ OR ‘pharmaceuticals’ OR ‘antibiotics’ OR ‘PPCPs’ OR ‘substance abuse’ OR ‘illicit drugs’) AND (‘India’)) NOT (DOCUMENT TYPES: (Review OR Meta-Analysis))

Table 2 | PICO elements and description

PICO element	Description (Applied to this study)
P (Population)	Wastewater as a public health surveillance tool for communities in India
I (Intervention/ Exposure)	Wastewater-based epidemiology (WBE)/wastewater surveillance for tracking community-level diseases/health
C (Comparison)	Traditional epidemiological methods (clinical testing, surveillance data, clinical isolates, or resistance network data)
O (Outcome)	Any form of comparison of wastewater surveillance results with epidemiological trends

the review. A total of 1,025 studies were identified from the database search. The literature search identified records from PubMed ($n = 351$), Scopus ($n = 347$), and Web of Science ($n = 327$). Out of which, 397 duplicate studies were removed. Six hundred and twenty-eight articles were further screened for the relevant title and abstract. Only 94 articles were fully assessed for their eligibility to be included in the review. Finally, 42 studies were found to be relevant following PICO (Figure 1). Although WBE-related activities surged in India during the COVID-19 pandemic, wastewater surveillance in the early 2000s had been successful in detecting the polio virus 3 months before the first reported case of poliomyelitis (Deshpande Jagadish *et al.* 2003). However, almost 90% of WBE studies in India occurred in and after 2020, reflecting how COVID-19 sparked WBE research in India. In total, 31 out of the 42 studies were related to the prevalence of COVID-19 in the population. Out of the remaining 11 WBE studies, 3 focused exclusively on AMR. The other 8 studies focused on infectious diseases, including enteric diseases and polio, except for one study on human gut microbiota. Overall, WBE studies in India have mainly targeted a group of infectious diseases and AMR. Details regarding the disease/biomarkers investigated and wastewater sampling protocols from the reviewed literature are shown in Supplementary

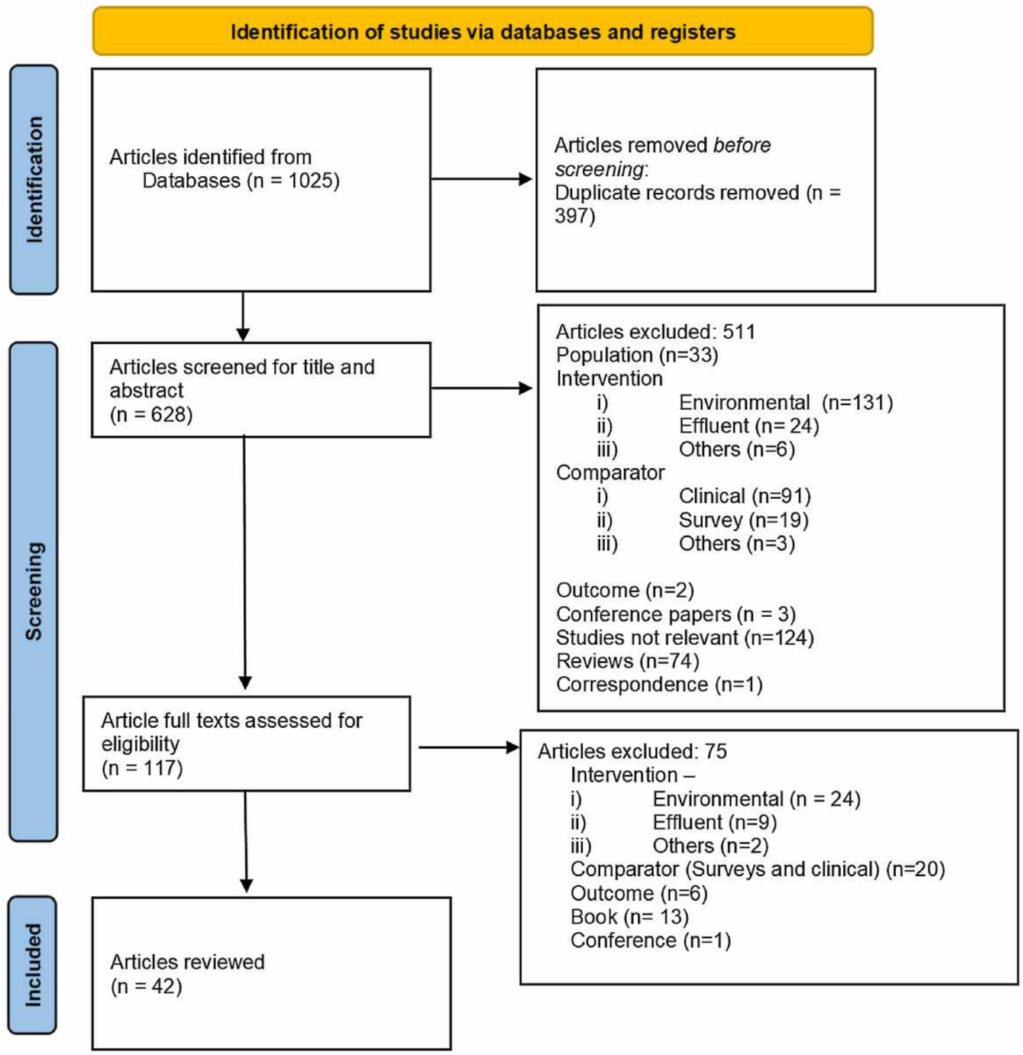


Figure 1 | PRISMA rable.

Table S1, whereas sample processing and analytical pipeline are summarized in Supplementary Table S2. A biomarker is defined as a measurable characteristic indicative of normal biological or pathological processes or responses to an exposure or therapeutic intervention (Strimbu & Tavel 2010).

3.1.2. Geographical coverage of WBE studies

WBE studies have been conducted for wastewater samples collected from several Indian states and union territories (UTs), including Delhi, Bihar, Jharkhand, Madhya Pradesh, Assam, Gujarat, Maharashtra, Karnataka, Rajasthan, Uttar Pradesh, Uttarakhand, Telangana, Kerala, West Bengal, Bihar, Chhattisgarh, Madhya Pradesh, Tamil Nadu, Manipur, and Arunachal Pradesh. Most studies have collected samples from Class 1 and 2 cities and towns. Cities/towns are classified as Class 1 and 2 based on their population. Class 1 cities have more than 100,000, whereas Class 2 cities have populations between 50,000 and 99,999 (Shaban *et al.* 2020). WBE studies served populations ranging from <1,000 (academic campus) to over 11 million people in a Class 1 city (Bengaluru) (Nag *et al.* 2022; Lamba *et al.* 2023). However, several studies did not explicitly report the size of the catchment population. Locations from which wastewater was collected include centralized and decentralized wastewater treatment plants, pump stations, hospitals (building-scale), and open/closed drains.

3.1.3. Sampling strategies

All studies except six employed a grab sampling methodology. The six studies performed manual discrete grab sampling and pooled them later to make composite samples. However, one study used the Moore swab technique, a passive composite sampling method, in addition to grab sampling in hospital settings and reported a higher detection of analytical targets than grab sampling (Uzzell *et al.* 2024). Moore swab is a sampling technique where a gauze pad tied with a string is suspended in wastewater (Rafiee *et al.* 2021). Most studies have collected wastewater samples in the morning; however, only one study conducted the recommended 24-h profiling of analytical targets before performing grab sampling to know the peak fecal loading times (Kopperi *et al.* 2021; Li *et al.* 2021b; Augusto *et al.* 2022). Only one study (Chaudhuri *et al.* 2023) reported in-sewer residence time of wastewater, which was 2–4 h, a critical parameter to assess the in-sewer analytical target stability. Only 3 studies (Purohit *et al.* 2017; Talat *et al.* 2023; Acheampong *et al.* 2024) out of 42 studies conducted were on WBE in rural settings. Most studies transported the collected grab samples to the laboratory on ice to maintain sample integrity, which is often required in WBE studies. Samples were stored under refrigerated conditions until processing. The sampling frequencies varied across the reviewed studies, ranging from hourly, daily, weekly, biweekly, monthly, bimonthly, to yearly sampling, with weekly sampling being the most common strategy. In several studies, sampling frequencies were unspecified. The total number of samples collected varied across studies, ranging from fewer than 10 samples to roughly 4,900 samples in longitudinal studies (Supplementary Table S1).

3.1.4. Disease and biomarker investigated

The following subsections provide an overview of the utility of WBE in biomarker or disease surveillance in India.

3.1.4.1. COVID-19 surveillance. Many COVID-19 studies have employed the heat inactivation technique as a laboratory biosafety procedure before processing wastewater samples. Mostly, two viral concentration methods, polyethylene glycol (PEG) precipitation and ultracentrifugation, have been used to detect SARS-CoV-2 RNA in wastewater. Seven studies related to COVID-19 have conducted genome sequencing to detect SARS-CoV-2 variants circulating in communities. The N (nucleocapsid), E (envelope), S (spike), ORF1ab, and *RdRp* genes were the most common analytical targets across reviewed studies. Most of the reviewed studies employed reverse transcription quantitative polymerase chain reaction (RT-qPCR) as the dominant analytical method. Digital PCR, which offers superior sensitivity and capability to perform absolute quantification without the need for standard curves, was used in only one study (Kumar *et al.* 2023b). Whole-genome sequencing was employed in studies targeting SARS-CoV-2 variant identification. Kumar *et al.* (2023a) incorporated a positive control (TaqPath™ COVID-19 Control), a negative control (extraction run spiked with MS2), and no template control with each batch, whereas Chakraborty *et al.* (2021) employed EURM-019 as an external positive control and endogenous RNase P from buccal swabs as an internal extraction control. Another study employed Bacteriophage Phi6 as an internal process control to assess recovery of enveloped RNA viruses from wastewater samples (Wani *et al.* 2023). SARS-CoV-2 viral load varied from below the detection limit to up to 10^6 genome copies/L of wastewater.

Several studies have demonstrated the early warning capability of WBE in India for COVID-19 by detecting SARS-CoV-2 and its variants in community wastewater well ahead (~14–20 days) of clinically reported data (Arora *et al.* 2022; Lamba *et al.* 2023). As noted elsewhere (Fontenele *et al.* 2021), studies conducted in India have also reported novel variants of the SARS-CoV-2 virus in wastewater, which were not detected in clinical datasets (Dharmadhikari *et al.* 2022; Rajput *et al.* 2023). WBE studies have reported several SARS-CoV-2 variants circulating in Indian communities, including lineages B.1.617, BA 2.75, XBB.1.16, and XBB.1.16.1. One study detected silent waves in the post-omicron period, with the XBB variant detected in wastewater 130–253 days before the clinical reporting (Rajput *et al.* 2024), indicating that WBE could be a leading indicator in detecting genomic variants when clinical machinery is strained. Studies found a positive correlation between clinical COVID-19 cases and WBE-generated SARS-CoV-2 surveillance data. One study reported a significant difference in SARS-CoV-2 virus load in the wastewater, with the highest concentration in summer, followed by monsoon and winter seasons, reflecting the seasonality in virus transmission within communities and the impact of wastewater dilution in the monsoon season (Kadam *et al.* 2024). One study has reported a high rate of COVID-19 infection in communities with high population density (Chakraborty *et al.* 2021). Tharak *et al.* (2022) have reported a rise in SARS-CoV-2 virus load in wastewater during the first and second waves of the COVID-19 pandemic, in agreement with the temporal variation in clinical cases.

3.1.4.2.Enteric pathogens and other infectious disease surveillance. The studies conducted in the early 2000s used wastewater surveillance as a tool to track wild poliovirus circulating in communities and understand the effectiveness of polio vaccination efforts (Deshpande Jagadish *et al.* 2003; Chowdhary & Dhole 2008). The poliovirus processing pipeline included virus isolation using cell cultures, followed by poliovirus typing and differentiation using neutralization tests, enzyme-linked immunosorbent assays (ELISA), and RNA probe hybridization with genome sequencing. Deshpande <span dataode-nwrap=$132#Jagadish *et al.* (2003) demonstrated that wastewater surveillance detected wild poliovirus 3 months before the first reported case of paralytic poliomyelitis. Apart from the early poliovirus surveillance studies, a few focused on other enteric infectious diseases, including typhoid. *Salmonella Typhi* (causative organism of typhoid) positivity rates of 7.50 and 15.28% were observed in grab samples and Moore swabs collected from open drains in Vellore, and wastewater positivity was correlated with seroincidence. Another study reported an approximately 40% positivity rate of enteroviruses correlating with serotypes of clinical stool samples (Tiwari & Dhole 2018).

Another study discovered a plethora of gut microbiota in wastewater collected from 21 Indian cities using the 16S rRNA sequencing technique and found *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* to be the dominant microbiota across study sites. In addition, the study indicated that the gut microbiome-human disease association can be useful in predicting population-level diseases, including diabetes, anemia, and obesity (Singh *et al.* 2023). This study found a higher microbial diversity and richness in urban regions compared with suburban and rural regions.

3.1.4.3. AMR surveillance. Besides pathogen surveillance, three studies have exclusively investigated the prevalence of antimicrobial resistance (AMR) in communities using wastewater fingerprinting, while one study focusing on microbiota also included antibiotic resistance genes (ARGs). Only those WBE studies related to AMR have been included in this study that compared wastewater data to either clinical isolates (Purohit *et al.* 2017) or the resistance network database (Prieto Riquelme *et al.* 2022; Madhukar *et al.* 2024). Culture-based antibiotic susceptibility testing coupled with PCR was employed in the study focusing on phenotypic characterization and genetic identification of resistant isolates (Purohit *et al.* 2017). Metagenomic sequencing was performed in studies with a focus on tracking the resistome of surveilled communities (Prieto Riquelme *et al.* 2022; Madhukar *et al.* 2024). One study conducted in 21 cities of India found 182 ARGs spanning 12 distinct classes. The general resistance trend was: aminoglycoside > beta-lactam > fluoroquinolone > macrolide > vancomycin > fosmidomycin > tetracycline. ARGs exhibited spatial variability, with the north and northeast regions showing higher macrolide, aminoglycoside, and chloramphenicol resistance, while the central region exhibited higher multidrug, macrolide, and imipenem resistance (Singh *et al.* 2023). However, another study in a southern Indian metropolitan city found that the macrolide class of antibiotics driven by 23S rRNA mutations exhibited the highest resistance at 40.1%, followed by aminoglycosides (16S rRNA) at 24.4%, tetracyclines (16S rRNA) at 11.3%, and lincosamides (23S rRNA) at 6.7% (Madhukar *et al.* 2024).

3.1.5. Methodological gaps

There has been no comprehensive study to test the in-sewer stability of investigated biomarkers, including SARS-CoV-2 viruses and AMR genes, under local conditions. Previous studies have shown a substantial reduction of ARGs, more than 50%, in sewer conveyance (Li *et al.* 2021a). WBE studies have been more biased toward urban settings, with only a few studies in rural settings in India, which need more focus to achieve equitable health outcomes. Moreover, reviewed WBE studies in India have overlooked its potential to address the need to monitor the prevalence of NCDs, a major contributor to the national burden of diseases, as discussed in the following sections. On the other hand, China, with a less than 50% sewered network coverage rate in urban built-up areas, has progressed much in WBE in the space of drug and infectious disease monitoring (Zhang *et al.* 2022; Xu & Xu 2023).

Although WBE studies spanned wide geographical regions of India, the depth of coverage was not uniform and equitable. Methodological heterogeneity existed across WBE studies conducted in India, limiting direct comparison of findings between studies. Heterogeneity arose from variability in sampling methods (grab, composite, and passive), sampling frequencies, sample processing workflows, virus concentration methodologies, and analytical techniques. Most studies did not include population normalization biomarkers or process recovery controls, potentially introducing substantial uncertainty in understanding spatio-temporal variability in disease trends across the country. Wastewater dilution due to rainfall (e.g., in open drain sampling) and transient population flux could result in incorrect estimations if not corrected with fecal markers. Furthermore, differences in sewer connectivity and climatic conditions could introduce bias in inter-study comparisons. Several studies were retrospective rather than real-time operational. Only 13 studies provided prospective early warning outputs. However, out of 13 studies, only one (Chaudhuri *et al.* 2023) translated WBE data into a real-time, publicly available dashboard with outcomes actively integrated into public health decision-making systems. Only one study explicitly claimed their study as a proof-of-concept WBE study in India (Kumar *et al.* 2020). Correlations with clinical findings were not statistically robust in several studies, with lead times not standardized (days vs. weeks), making cross-study comparison difficult. In addition, confounding factors such as COVID-19 testing rates, reporting delays, and catchment uncertainty were not consistently addressed.

3.2. Potential of WBE in India

The following subsections discuss the potential application of WBE in India in population health surveillance, considering the country's disease burden, regional epidemics, and local mass gatherings and festival events, within the context of sanitation infrastructure. However, it should be noted that the following discussions are not based on the findings of the systematic review; rather, they provide a perspective on potential applications of WBE in India.

3.2.1. GBD 2021 report and WBE in India

As per the GBD 2021 report (IHME 2024), multiple behavioral, environmental, and metabolic risk factors contribute to the disease burden in India (Table 3). Overall, behavioral risk factors (26.61% of total DALYs) contribute the most to India's disease burden, followed by environmental (19.6%) and metabolic risk factors (14.41%) (IHME 2024). Population exposure to contaminated water, lack of sanitation and hygiene, air pollution, and malnutrition are driving risk factors for the prevalence of CMNN diseases. Learning from the COVID-19 pandemic pushes the need for a rapid public health surveillance tool, such as WBE, particularly in the early phase of new communicable disease progression, when the clinical machinery is not yet ready (Bowes *et al.* 2023a). In the event of any future pandemic, WBE, a silent sentinel of community health, can guide when to implement intervention measures and ease them without causing a significant economic strain.

Although respiratory infections and tuberculosis are still the leading causes of DALYs per hundred thousand population in India, NCDs contributed the most to total DALYs in 2021 (Figure 2).

According to the World Economic Forum, the economic burden on India due to NCDs and mental health may rise to \$4.58 trillion by 2030 (Bloom *et al.* 2014). High blood glucose, high fasting blood pressure, high low-density lipoprotein levels, high body mass index, and kidney dysfunction are the most common metabolic risk factors for the NCD burden. Substance abuse and diet are the prominent behavioral risks for developing NCDs, whereas air pollution, lead exposure, and occupational exposure are the main environmental risk factors for developing NCDs (IHME 2024). Traditional approaches to NCD monitoring in India include population-based surveys with a set of questionnaires, physical measurements (height, weight, and blood pressure), and biochemical measurements (fasting blood glucose and urinary sodium in spot urine samples) (NCDIR 2017), which may not provide timely information on risk factors contributing largely to NCDs. The prospect of WBE in near real-time monitoring of risk factors associated with diseases is summarized in Table 3. Human fecal and urinary biomarkers for almost 90% of the risk factors contributing to disease burden have been either tested for WBE or are potential candidates (Table 3). However, many biomarkers listed in Table 3 have been tested in high-income settings. The detection and stability of these biomarkers need to be validated in the Indian context, particularly in regions with high temperatures, intermittent flow, and non-sewered sanitation infrastructure. Substance use, including tobacco, alcohol, and drugs, collectively constitutes the largest risk factor (>1,500 DALYs per 100,000 population) for the burden of diseases in adults aged between 15 and 49 (IHME 2024). There have been several reports of drug abuse and related self-harm incidents among college and university youths in India (Desai *et al.* 2021; Sahu *et al.* 2022). The national survey on the magnitude of substance use in India indicated that alcohol, cannabis, and opioids are the top three commonly used substances in India, with the prevalence of alcohol being the highest (14.6%; 10–75 age group) (Ambekar *et al.* 2019). Other substances used by Indian drug users include sedatives, inhalants, stimulants, including cocaine and amphetamine-like substances, and hallucinogens. The prevalence of opioid use in India is almost three times the global prevalence. On average, 775 people died each year during 2016–2019 in India due to drug overdose deaths (MSJE 2021). In addition, self-medication and potential misuse of pharmaceuticals are common in India due to over-the-counter sales of regulated pharmaceuticals without prescriptions, failure of India's drug regulatory framework, and unnecessary fixed-dose combination drugs (Shankar *et al.* 2016; McGettigan *et al.* 2017; Marathe *et al.* 2020). The issue of unnecessary fixed-dose combination drugs has been reported elsewhere, such as the antibiotics meropenem and sulbactam. They are sold as a combination drug and show no additional advantage in combination, but they do induce toxic reactions and foster drug resistance (Ahmad *et al.* 2016). The near real-time surveillance of prescription and illicit drug use using high-resolution WBE at the neighborhood level could assist the relevant public health authorities in targeted interventions to minimize drug overdose deaths. Moreover, any discrepancy observed between WBE data and drug prescription dispensing and sales data may help understand drug misuse or

Table 3 | Status of WBE in monitoring GBD risk factors

S. No.	Risk factors	Diseases	Biomarkers relevant to WBE	References
1	Malnutrition[a]	RI, MN, ONC, EI, ND, OI, NTDM	1. Anemia – *Hepcidin*[b] 2. Vitamin A deficiency[b] – *retinoic acids (RAs) and 4-oxo-RAs*	Yeung *et al.* (2021), Kemna *et al.* (2007)
2	Tobacco[a]	RI, CVD, CR, NP, MSD, UI, DCKD, DD, SOD, NLD, SHV, TI	*8-iso-prostaglandin F2α*[c], Tobacco consumption specific biomarkers[c] including Nicotine, Cotinine, Hydroxycotinine, Anabasine, Anatabine, *Gut microbiota*[c], including *Neisseria, Desulfovibrio, Megamonas, Blautia, Fusicatenibacter, Granulicatella and Enterococcus*	Castiglioni *et al.* (2015), Zheng *et al.* (2020), Ryu *et al.* (2016b), Wu *et al.* (2023)
3	High alcohol use[a]	RI, CVD, NP, UI, DCKD, DD, NLD, SHV, TI, SU	Ethyl Sulfate[c]	Ryu *et al.* (2016a)
4	Dietary risks[a]	RI, CVD, NP, DCKD,	Enterodiol, Enterolactone, and Proline betaine (biomarkers for plant-based diet[c]); *gut microbiota*[c] involved in phytoestrogen metabolism, including *Bifidobacterium, Blautia, and Romboutsia*	Choi *et al.* (2020), Bowes *et al.* (2023b)
5	Low physical activity[a]	RI, CVD, NP	*Endogenous urinary proteome*[b]	Sampson *et al.* (2014)
6	Drug use[a]	NP, DD, SHV, OI, SU, HAS	Illicit drugs[c] including benzoylecgonine (cocaine), methamphetamine, MDMA, 6-acetyl morphine (heron)	Zuccato *et al.* (2008)
7	Childhood sexual abuse/bullying[a]	MD, SU	No specific biomarker is available	
8	Intimate partner violence[a]	MD, SHV, HAS	No specific biomarker is available	
9	Unsafe sex[a]	NP, HAS	Chlamydia trachomatis[c] (biomarker for Chlamydia), Neisseria gonorrhoeae[c] (biomarker for Gonorrhea), Treponema pallidum[c] (biomarker for Syphilis), HIV[c] (a biomarker for AIDS); HIV drugs[c], including lamivudine	Zhao *et al.* (2024), Hou *et al.* (2020)
10	Air pollution[d]	RI, CVD, MN, CR, NP, ONC, EI, DCKD, SOD, OI	1. Occupational PAH – Hydroxyl derivatives[c], including 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 9-hydroxyfluorene, 9-hydroxyphenanthrene, 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene 2. Occupational benzene – *N*-Acetyl-*S*-(phenyl)-*L*-cysteine[c] 3. Asthmagens, PM_{10} and $PM_{2.5}$-Salbutamol[c]	Fattore *et al.* (2016), Kumar *et al.* (2022b), Styszko *et al.* (2025)
11	WASH[d]	RI, EI	Enteroviruses[c], respiratory viruses[c]	Chacón *et al.* (2021), Fantilli *et al.* (2023)
12	Non-optimal temperature[d]	RI, CVD, CR, DCKD, SHV, TI, UI	Increase in temperature[c] – Acesulfame (a marker for consumption of sweetened drinks), atenolol (a marker of worsening cardiovascular conditions), naproxen (a marker of worsening pain)	Phung *et al.* (2017)
13	Others[d] (Lead/ Radiation)	CVD, MD, NP, DCKD	Lead exposure – diethyl lead[b] and ethyl lead[b]	Markosian & Mirzoyan (2019)
14	Occupational risk[d]	CR, NP, MSD, UI, SOD, TI	1. Occupational PAH – Hydroxyl derivatives[c], including 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 9-hydroxyfluorene, 9-hydroxyphenanthrene, 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene	Styszko *et al.* (2025), Kumar *et al.* (2022b), Fattore *et al.* (2016), Pan *et al.* (2024)

(*Continued.*)

Table 3 | Continued

S. No.	Risk factors	Diseases	Biomarkers relevant to WBE	References
			2. Occupational benzene – *N*-Acetyl-*S*-(phenyl)-*L*-cysteine[c] 3. Occupation trichloroethylene – *N*-Acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine[c] 4. Asthmagens, PM_{10} and $PM_{2.5}$-Salbutamol[c] 5. Occupational arsenic – monomethylarsonic acid[b], dimethylarsinic acid[b], monomethylarsonous acid[b], and dimethylarsinous acid[b] 6. Occupational formaldehyde – Thioproline[b] and Thioprolinyl glycine[b]	
15	High fasting plasma glucose[e]	RI, CVD, DCKD, SOD, NLD, NP	Antidiabetic drugs, including metformin[c]	Zheng *et al.* (2023)
16	High body mass index[e]	RI, CR, MSD, DCKD, SOD, NLD, NP, CVD, DD	1. Gut microbiome[c], including Ruminococcus_E, Agathobacter, Fusicatenibacter, Anaerobutyricum, Blautia_A and Neisseria 2. Gut microbial co-metabolites[b], including 4-cresyl sulfate, 3-methylhistidine, 2-hydroxybutyrate, phenylacetylglutamine (endogenous biomarkers) 3. Obesity drugs[b], including semaglutide	Wu *et al.* (2024), Elliott *et al.* (2015), Knop *et al.* (2023)
17	High blood pressure[e]	CVD, DCKD	1. Beta-blocker drugs[c], including metoprolol, metoprolol acid, atenolol, sotalol, and nadolol, Cystatin C, cardiac troponin I (Proposed endogenous biomarkers) 2. Calcium channel blockers[c] like amlodipine, nifedipine (nornifedipine), diltiazem, *N*-Desmethyl-Diltiazem 3. Angiotensin-converting enzyme inhibitor[c], including Lisinopril 4. Angiotensin II receptor blockers[c], including losartan, telmisartan, valsartan 5. Diuretics[c] like Hydrochlorthiazide	Hou *et al.* (2023), Shao *et al.* (2023), Gao *et al.* (2021), Galani *et al.* (2021)
18	High low-density lipoproteins[e]	CVD	Lipid regulator drugs[c], including gemfibrozil, atorvastatin, 2-hydroxy-atorvastatin, Rosuvastatin	Galani *et al.* (2021)
19	Kidney dysfunction[e]	CVD, MSD, DCKD	*Cystatin C*[b], *Uromodulin*[b], *C-reactive protein*[b]	Rice & Kasprzyk-Hordern (2019)
20	Low bone density[e]	UI, SHV, TI	*N-telopeptide (NTx)*[b], Bisphosphonate drugs, including Alendronate, Ibandronate, and Zoledronic acid	Eyre (1997), Lin (1996)

RI, Respiratory infections & TB; CVD, Cardiovascular diseases; MN, Maternal and neonatal diseases; CR, Chronic respiratory diseases; MD, Mental disorders; NP, Neoplasms; MSD, Musculoskeletal diseases; ONC, Other non-communicable diseases; UI, Unintentional injuries; EI, Enteric infections; DCKD, Diabetes & CKD; DD, Digestive disorders; OCO, Other COVID-19 related outcomes; NLD, Neurological disorders; SOD, Sense organ diseases; ND, Nutritional deficiencies; SHV, Self-harm & violence; TI, Transport injuries; OI, Other infectious diseases; NTDM, Neglected tropical diseases & malaria; SU, Substance use disorders; HAS, HIV/AIDS & STIs; SD, Skin and subcutaneous diseases.

[a]Behavioral risk.

[b]Detected in urine but not yet tested for WBE/Potential WBE markers.

[c]Tested to WBE.

[d]Environmental/occupational risk.

[e]Metabolic risk; Endogenous biomarkers are listed in *italics*.

illegal use of pharmaceuticals, provided the wastewater catchment is understood, target drugs show in-sewer stability, and the prescription sales data is reliable (Boogaerts *et al.* 2021).

Communities' exposure to chemicals is the major driver for the development of NCDs (Zarean & Poursafa 2019). The disease burden in India attributable to chemical exposure has been estimated for only a few types of chemicals, including metals, silica, polyaromatic hydrocarbons, and volatile organic compounds (IHME 2024). Overall estimation of the burden of diseases due to chemical exposure may be underestimated because several EDCs, including pesticides, plasticizers,

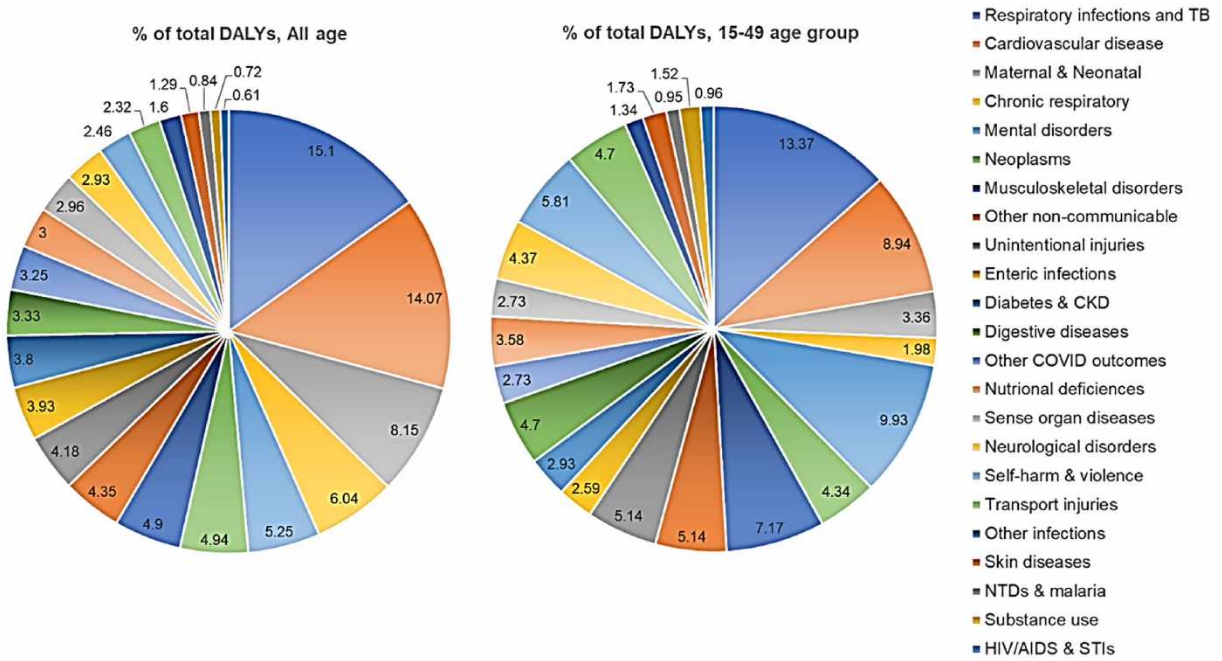


Figure 2 | Percentage of total DALYs contributing to the burden of diseases in India, 2021 (IHME 2024).

per- and polyfluoroalkyl compounds, and polychlorinated biphenyls, have not been included as GBD risk factors. For instance, lethal pesticide poisoning is of significant concern in the Indian population, particularly farmers, who are exposed to pesticides used in agriculture (Karunarathne *et al.* 2021).

A few studies in India have attempted to estimate chemical exposure by measuring chemical residues in human secretions, including blood, urine, and milk (Guo *et al.* 2011; Anand *et al.* 2021), or indirectly, by monitoring them in dietary intake (Chakraborty *et al.* 2022). A nationwide human biomonitoring program in India, similar to the U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES), is essential to estimate pan-India exposure, providing baseline data on chemical exposure to the Indian population over time (Stanfield *et al.* 2024). WBE could complement human biomonitoring efforts to provide community-wide chemical exposure assessments.

3.2.2. Tropical vector-borne diseases – endemic phenomena

Although vector-borne diseases do not fall under the top 20 burden of disease in India (Figure 2), they have become epidemic in several states of India, imposing a significant strain on India's healthcare system every year (Naik *et al.* 2023). Increases in temperature and changes in precipitation patterns due to climate change, along with the development of urban slums with poor sanitation, have escalated the infection geography of vector-borne and zoonotic diseases in India in the last two decades.

India's country-specific common vector-borne diseases include dengue, malaria, kala-azar, chikungunya, Japanese encephalitis, and Filaria (Gaurav *et al.* 2024). From 2019 to 2023, there has been an overall upward trend in the number of reported cases of dengue infection, except in 2020, when the number of reported cases significantly fell due to overlapping clinical and laboratory features of dengue and COVID-19, resulting in difficulty in diagnosing and differentiating the causes (Yan *et al.* 2020; Sasmono & Santoso 2022; Mondal 2023; NCVBDC 2023). To minimize the spread of the dengue virus, there is a need for robust and real-time surveillance for early detection and more efficient disease management. The detection of Chikungunya and Dengue viruses in wastewater has been recently reported in India (Stockdale *et al.* 2023), indicating the potential of WBE in monitoring vector-borne diseases at the national scale (McCall *et al.* 2020; Sílvia *et al.* 2023; Wolfe *et al.* 2024). Also, WBE can act as a relatively fast diagnostic tool to identify the causative agent where infectious diseases exhibit overlapping clinical symptoms.

3.2.3 Measuring the success of regulatory restrictions

WBE can play an instrumental role in tracking the effectiveness of regulatory bans and restrictions on the manufacturing, sale, and consumption of chemicals and substances impacting public health, which otherwise would have been difficult to monitor (Adhikari *et al.* 2022; Tscharke *et al.* 2023; Verhagen *et al.* 2024). The consumption of illegal drugs, pesticides, and other chemical substances hazardous to public health could be easily monitored by WBE. For example, tracking alcohol consumption markers in community wastewater of a few Indian states, including Bihar, Gujarat, Mizoram, and Nagaland, where there is a complete ban on the sale and consumption of alcohol, could help regulators understand the efficacy of the ban (Schess *et al.* 2023). Recently, a WBE study successfully evaluated the usage of regulated antimicrobials, triclosan and triclocarban, in U.S. communities following their ban by the U.S. Food and Drug Administration (FDA) from thousands of over-the-counter (OTC) personal care products (Adhikari *et al.* 2022).

3.2.4. Estimation of population health during festivals and mass events

WBE can be extremely useful in tracking the population's health during festivals in India when people are potentially exposed to hazardous chemicals, including heavy metals and hydrocarbons, when they sprinkle colored powders (Gulal) over each other or light fireworks marking celebrations. The surveillance of urinary biomarkers in wastewater indicative of population exposure to chemicals during and after the festival may help public health agencies estimate their impact on public health.

The overcrowding at religious or non-religious gatherings potentially escalates the risk of the spread of infectious diseases. India is known for the world's largest religious mass gathering known as 'Kumbh Mela', which occurs every 3 years for a couple of months in India, in four cities of India on the banks of the holy rivers (Memish *et al.* 2019). India is also known for its most popular sport, cricket, with millions of followers. The country organizes many domestic and international cricket matches every year, attracting more than a hundred thousand people to attend each event. Although the government has been proactive in managing the huge crowds, providing medical assistance, and maintaining sanitation, there is a need for active public health surveillance during such events to prevent an epidemic. The planned surveillance of collective urine and feces from public toilets and wastewater treatment plants, and monitoring of open drains during and after the event, may help the public health authorities to prepare for any potential disease outbreak.

3.2.5. Status of sanitation infrastructure in India

To implement WBE nationally to address the above-mentioned public health challenges, knowledge of sanitation infrastructure across the nation is imperative. In India, domestic wastewater and fecal sludge collection and treatment units have been built under several sanitation-driven national schemes and programs, including the Swachh Bharat Mission (SBM), Atal Mission for Rejuvenation and Urban Transformation (AMRUT), Smart Cities Mission, and the National Mission for Clean Ganga (NMCG). The Central Pollution Control Board (CPCB), a statutory organization of India, published the national urban wastewater treatment inventory in 2021 after consultation with the state pollution control boards and pollution control committees (CPCB 2021). The SBM website also has an online dashboard displaying the state-wide number of wastewater and fecal sludge treatment plants. As per the CPCB report, 1,631 wastewater treatment plants (including proposed ones) cover 35 states/UTs in India. However, the installation capacity of wastewater treatment plants is not equitable throughout the nation. Around 86% of the total installed wastewater treatment plants are concentrated in 10 states, including Maharashtra, Gujarat, Delhi, Uttar Pradesh, Karnataka, Haryana, Madhya Pradesh, Punjab, Tamil Nadu, and Rajasthan. In contrast, several states and the UTs, including Arunachal Pradesh, Andaman & Nicobar Islands, Lakshadweep, and Meghalaya, have not installed centralized wastewater collection systems (CPCB 2021; MOHUA 2022). In addition, the utilization of existing infrastructure is only 75% of operationalized treatment capacity, indicating a lack of extensive sewerage conveyance systems, including sewer lines and household sewer connections (CPCB 2021). Nationwide implementation of WBE in urban India cannot be successful and inclusive if non-sewered regions are overlooked. Open/closed drains and surface water bodies can be sampled in non-sewered regions to provide more insight into public health. This review indicated 11 studies (Supplementary Table S1) sampled either surface water bodies or open/closed drains to conduct wastewater surveillance studies. Globally, WBE studies have dominated in sewered regions or cities with centralized wastewater treatment plants (Gonçalves *et al.* 2022), leading to established sampling and analytical protocols while creating a wide knowledge gap in their implementation in decentralized or onsite wastewater collection systems.

The Department of Drinking Water and Sanitation launched SBM Grameen (SBM-G) in rural areas in 2014 with a mission to make India Open Defecation Free (ODF), with subsequent SBM-G 2.0 in 2021. ODF villages with solid and liquid waste management facilities are termed ODF-plus villages (DDWS 2024). As of now, there are 564,124 ODF-plus villages in India (DDWS 2024). All states/UTs, except for Andaman & Nicobar Islands, Manipur, Meghalaya, and Nagaland, declared more than 90% of their villages ODF-plus. Andaman &Nicobar Islands, Meghalaya, and Nagaland have more than 50% ODF-plus villages; however, Manipur has only 3% ODF-plus villages (DDWS 2024). ODF-plus villages have their onsite sanitation infrastructure, including community septic tanks, community soak pits, and fecal sludge treatment plants that can serve as potential sampling points from the WBE perspective.

Overall, sanitation infrastructure across India indicates a promising future for WBE-related activities in India.

4. CONCLUSIONS AND FUTURE RECOMMENDATIONS

This study concludes that although COVID-19 sparked WBE-related efforts in India, its broader potential has not yet been fully realized, particularly in the domain of tracking risk factors for NCDs. Findings of the systematic review revealed that the WBE studies were limited to infectious diseases and AMR surveillance. WBE studies have largely concentrated in urban centers, underscoring the need for an equitable approach that encompasses suburban and rural regions. Methodological heterogeneity and confounding factors across studies limit inter-study comparisons and the drawing of epidemiological inferences. Although several studies demonstrated correlations between WBE-derived data and clinical data, only one study integrated wastewater data into public health decision-making, indicating a gap in translating WBE data into actionable public health outcomes. Based on the insights gained from the systematic review, this study provides the following recommendations for the sustainable, long-term deployment of WBE in India.

4.1. Prioritize diseases/health conditions and identify target biomarkers

Before laying out the WBE program, there is a need to identify India's public health priorities, which could be local epidemics like dengue or key health indicators resulting in a significant burden of diseases. In addition, public health priorities may also differ across states. The Indian Council of Medical Research (ICMR) has developed the priority pathogen list in consultation with clinicians, laboratory experts, and epidemiologists across the country, comprising pathogens associated with acute febrile illness, acute encephalitis syndrome, acute respiratory illness, and acute diarrheal disease (ICMR 2025). In addition, the World Health Organization (WHO), in collaboration with the Department of Biotechnology (DBT), Government of India, has developed the Indian priority pathogen list with priorities classified as critical, high, and medium for AMR. The Enterobacteriaceae (*Klebsiella pneumoniae* and *Escherichia coli*) and non-fermenting bacteria (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) are classified under the critical priority list (WHO 2019). In some cases, for example, monitoring community consumption of novel psychoactive substances within a rapidly emerging drug market, suspect screening of wastewater using high-resolution mass spectrometry may help in prioritizing target analytes (Lee & Oh 2023). For prioritizing targets for NCD surveillance, clinical and health survey data may be consulted.

The second step should be to identify human urinary and/or fecal biomarkers specific to the prioritized disease(s). The potential biomarker candidates suitable for WBE should be unique to human excretion, be amenable to detection, and be stable in wastewater (Gracia-Lor *et al.* 2016).

4.2. Strategize wastewater sample collection and transport in line with WBE objectives

Selection of suitable sampling locations, frequency, methodology, transportation, and analytical protocols is a fundamental requirement for optimizing the WBE workflow. The sampling location should be decided based on the type of sanitation infrastructure available and public health needs. The sampling frequency should depend on the key objectives of the project. Although the variance in the preliminary WBE data may help best decide the suitable sampling frequency, a weekly sample may be sufficient to screen for the presence of the target pathogen; however, at least three samples are required to establish wastewater trends over time (CDC 2023). If the study's objective is to inform policy and public health interventions to reduce harm from substance use, random sampling may not be representative of the actual community-scale drug use behavior. Global WBE studies have shown that substance use is subject to strong weekly and seasonal cycles (Humphries *et al.*

2016). The monitoring scheme should be evaluated considering the operational feasibility, logistics, and budget of the project.

The sample collection methodology could be either a grab or a composite; however, flow-proportional continuous autosampling has been considered the gold standard (Ort *et al.* 2010a, 2010b). To collect the best representative grab sample, 24-h profiling of the target pathogen should be done to determine the peak fecal loading times before selecting the sampling time window (Li *et al.* 2021b).

Refrigerated battery-operated autosamplers can perform 24-h composite sampling at a desired sampling interval at 4 °C, even in remote areas with limited access to electricity. However, they are expensive, may take several weeks of transit time to import from overseas to India, and there is a risk of theft in remote locations if left unattended. Locally manufactured non-refrigerated autosamplers are the alternative options, provided samples are collected on ice packs. Studies have shown that the stability of many chemicals is improved under refrigerated conditions (Lin *et al.* 2021). Passive sampling is recommended as an alternative to composite sampling in low-resource settings and where flow is not persistent. However, passive sampling devices are prone to saturation, and the measured analyte concentration may not be normalized to flow (Robins *et al.* 2022). Nevertheless, passive sampling may provide an early warning by detecting biomarkers in wastewater in advance of their detection in clinical settings. Autosampling devices are unsuitable for sampling thick sludges and highly concentrated liquids from septic tanks and pit latrines. Manual grab sampling at regular intervals can be done instead if automated sampling is not feasible.

Ideally, samples should be kept refrigerated while collecting and transporting to the laboratory, considering that the alarmingly high summer temperatures in several states of India may impact the target analyte stability (Lin *et al.* 2021). High temperature is anticipated to cause in-sewer and in-sample decay of urinary and fecal biomarkers, leading to incorrect WBE-derived estimations. However, several field-based pathogen testing kits have been developed for use in remote locations when transporting samples to the laboratory on time is not feasible (ARUP 2021). Future year-long WBE studies in regions of India with substantial seasonal temperature fluctuations should not directly compare inter-seasonal data unless the biomarker stability has been evaluated.

A WBE study is feasible if there is a good understanding of the catchment and dynamics of the wastewater network. WBE could be implemented initially in Class 1 cities with already established piped sewerage networks, before expanding to Class 2 cities and beyond. Implementing WBE can become challenging in ancient cities of India, where the sewer network map of the catchment is not readily available. However, geographic information systems (GIS) and remote sensing, in addition to field investigations, may help in identifying sewer networks.

4.3. Plan to archive wastewater samples

This study recommends setting up a national wastewater repository in India. Every district of India should plan to set up a wastewater storage facility. Archived samples from all districts should be barcoded, and all data should be accessible to the relevant authorities at the proposed National Wastewater Informatics Centre. Archived samples may be crucial from a future perspective to determine retrospective trends in disease prevalence and population exposure to chemicals and biological agents. Also, with improved analytical capabilities and sensitivities, there may be a need to re-analyze old samples to extract more information that was not foreseen. CDC recommends a minimum of three samples to detect wastewater trends over time. Australia's national wastewater monitoring program has also considered archiving samples collected during the census in its framework (O'Brien *et al.* 2019). Archiving weekly or biweekly samples may be more economically feasible due to limitations associated with storage space and refrigeration costs. However, optimal storage conditions may vary with target analytes. If the purpose of archiving is for both chemical and biological analytes, a compromise may be necessary to select the most appropriate storage condition. For archiving samples for illicit drug analysis, the addition of isotope-labeled standards is recommended either before storing samples or immediately after the samples have thawed if standards were not added before storage to compensate for analyte loss and transformation (McCall *et al.* 2016). Storing wastewater extracts of small volume (<1 mL) can be a cost- and space-effective way to archive samples instead of 50–100 mL volumes (Farkas *et al.* 2024). Moreover, minimal loss of viral RNA/DNA has been noticed in nucleic acid extracts compared with raw wastewater stored for 8–24 months, indicating wastewater extracts could be preferred if biobanking is intended for long-term storage (Farkas *et al.* 2024). Although archived samples may be useful for retrospective suspect and non-target screening with caveats, long-term storage of wastewater samples could offer numerous applications in chemical analysis.

4.4. Focus more on non-sewered areas

As of the 2021 National Family Health Survey, roughly 7.1% of people used toilets connected to a piped sewer network, whereas the majority of the population used toilets that are flushed into onsite containment systems (Jain *et al.* 2024). However, the sewered population (~100 million people) is still larger than in many developed countries where WBE is in operation. Open drains are more prevalent in rural communities, while closed drains are more common in urban settings (Jain *et al.* 2024). Implementing robust WBE with a similar intent and effort in sewered and non-sewered areas could help public health authorities channel resources to marginalized communities, thus reducing health disparities. However, extensive training, effort, and skilled capacity building are needed to implement WBE in non-sewered areas (Rochelle *et al.* 2023). Unlike defined sampling sites in sewered communities, unsewered sampling sites are variable and need validated standard operating procedures. Key challenges include selecting representative sample collection sites (pit latrines, septic tanks, open drains, fecal sludge decanting vehicles, or wastewater/fecal sludge treatment plants), overcoming sampling difficulties, ensuring adequate sample yields, and accurately estimating the contributing population to derive epidemiological estimations. The composition of fecal sludge varies temporally and spatially; therefore, collecting only one grab sample from one sampling point may not be representative. A representative sample of fecal sludge should include the scum, supernatant, and sludge layers collected at variable depths and times from the entire fecal sludge management chain, including collection (septic tanks), transportation (trucks carrying sludge to the emptying site), and treatment/disposal (fecal sludge treatment plant) (Velkushanova *et al.* 2021). The choice of sampling equipment depends upon various factors, including sludge thickness, depth, and accessibility. Fecal sludge sampling also triggers several health and safety (H&S) issues that need to be addressed, including inaccessible (cemented septic tanks) and structurally unstable sampling sites, potential exposure to toxic gas buildup during anaerobic digestion, and the risk of spillage of fecal material during sampling. A thorough H&S training is required before planning to lay the WBE pipeline. Appropriate personal protective equipment (PPE), including boots, masks, gloves, and outerwear, should be worn while performing wastewater sampling (ARUP 2021).

4.5. Perform the source apportionment study

Since the majority of the population is non-sewered, chemical and biological characterization of wastewater is recommended alongside the WBE data interpretation to understand the source apportionment of target analytes. For instance, open drains may receive inputs from human and/or animal waste or industrial discharge. Source tracking of the target marker should be prioritized. For accurate estimation of the population-level consumption of pharmaceuticals or illicit drugs in mixed sewer systems receiving both domestic and industrial inputs, unique human urinary or fecal metabolites should be selected as target analytes. In addition, genetic fecal markers and microbial source tracking markers (genetic fecal pollution diagnostics, GFPD), in parallel to genetic detection of pathogens, will help to characterize the origin of wastewater (Demeter *et al.* 2023). The added advantage of GFPD is that these markers can be analyzed retrospectively from archived samples, enabling robust quality control in data interpretation.

The microbial concentrations in wastewater may vary with flow and population flux. Normalization with a human fecal indicator, including Pepper mild mottle virus (PMMoV), could provide reliable spatio-temporal comparisons across sites. Several chemical markers, such as caffeine, ammonium, and sucralose, have also been investigated previously and could be used to normalize the target biomarker concentrations when the population contributing to the sampling location cannot be correctly estimated, particularly in non-sewered settings (Been *et al.* 2014; O'Brien *et al.* 2014; Senta *et al.* 2015). However, population markers should be locally validated before considering them in WBE-based estimations, as dietary preferences may vary considerably across sampling locations, particularly in a country-wide study.

4.6. Set up a decision threshold for NCD to inform public health decisions

To set up a decision threshold to inform public health decisions, baseline data will be needed. Longitudinal community wastewater sampling across the rural-to-urban gradient will be needed due to a lack of any existing WBE data for NCD. The resulting WBE-derived NCD prevalence data, normalized with population markers, should serve as the baseline. In addition, there should be a focus on monitoring NCD risk factors using wastewater monitoring. The WBE-derived data should be correlated with the clinical NCD data across regions. Once the baseline is established in coordination with the secondary data, a minimum threshold for WBE data should be decided. Once the wastewater signals statistically exceed that threshold, public health interventions could be triggered. The WBE data could complement the Government of

India's National Program for Prevention and Control of Non-Communicable Diseases (NP-NCD) as part of the National Health Mission (NHM) by identifying NCD hotspots across the country (MoHFW 2026).

The individual-level NCD screening executed by trained frontline medical and paramedical workers under the NP-NCD incurs a considerable recurring cost. According to the Ministry of Health and Family Welfare, total funds allocated for NP-NCD during the financial year 2024–2025 were around 1,400 crores (~170 million USD) across all states and UTs (MoHFW 2025). Although the WBE has globally proven to be a cost-effective public health surveillance tool, an India-specific cost comparison study should be conducted. Nevertheless, WBE should serve as a secondary surveillance layer, providing rapid, actionable data enabling the government to channelize its resources to NCD hotspots.

4.7. Encourage the public–private partnership (PPP) model

There is a need for partnerships for funding, implementation, and impact creation in the context of WBE. The setting up of a WBE pipeline requires generous capital funding. In addition to seeking grants from the Government of India funding bodies, institutions should also approach large corporates, multinational companies, and micro, small and medium enterprises (MSMEs) for corporate and social responsibility (CSR) funding. WBEs can easily attract CSR funding because of its socio-environmental outcomes. International organizations could also be contacted for WBE-related financing. The World Bank has launched a Pandemic Fund initiative to finance pandemic prevention and preparedness projects, particularly in low- and middle-income countries (Evaborhene 2023). The cost for conducting WBE in India for monitoring the SARS-CoV-2 virus has been estimated at $34 per sample, which includes sample collection, transportation, storage, and analysis-related costs (Hu *et al.* 2023). However, there is no existing literature on the cost analysis of WBE relative to conventional epidemiological surveillance in India. Although a U.S. study indicated the cost of WBE was 0.5% of conventional public health monitoring, the same cannot be applied in the Indian context (Driver *et al.* 2020). The manpower cost in India is significantly lower than in developed nations, potentially narrowing the comparative cost advantage of WBE over conventional surveillance tools. On the other hand, WBE studies in India would typically require considerable capital investments due to the need to import supplies, including analytical-grade standards, refrigerated autosamplers, and high-end instruments. Nevertheless, once the infrastructure is established, the recurring cost of WBE needs to be compared with the cost of conventional public health surveillance methodologies at a similar population coverage. In addition, the economic burden of public health due to the delayed detection and reporting of public health threats within the conventional surveillance framework needs to be considered to determine the long-term economic feasibility of WBE in India.

Active partnerships of the government with non-governmental organizations (NGOs) and private laboratories are needed to share wastewater samples and project methodology, deliver significant outcomes, and impact policy interventions. NGOs can act as a pressure group to convince the government to prioritize WBE research in the country. One such example is the success of the Precision Health platform, a multi-partner organization involving non-profit organizations and Government bodies that has taken a bold initiative in monitoring SARS-CoV-2 and other viruses in multiple cities of India (Chaudhuri *et al.* 2023). CryptoRelief, a non-profit organization, supported the Indian Institute of Technology Madras, a leading research institution in India, to develop a WBE research facility during the COVID-19 pandemic (CryptoRelief 2022).

4.8. Implement WBE at sentinel sites

Globally, WBE has played a crucial role in tracking infectious diseases at sentinel sites, including transport hubs (Ahmed *et al.* 2020), tourist spots (Sangsanont *et al.* 2023), hospitals (Gonçalves *et al.* 2021), academic institutions (Scott *et al.* 2021), and animal farms (Ahmed *et al.* 2024), by sending an early warning signal of potential outbreaks to concerned authorities, triggering a targeted public health response. In India, most of the sentinel sites, including airports, hospitals, and academic institutes, have onsite wastewater collection systems that could be leveraged for the WBE pipeline. It has been found that college student travel was linked to the local COVID-19 spikes (Mangrum & Niekamp 2022). There are 1,113 universities, 43,796 colleges, and 11,296 stand-alone institutions in India, with around 40 million students enrolled in 2020–2021 (Ministry of Education 2021). Implementing WBE paired with clinical testing at the academic catchment scale should help authorities keep universities open and avoid suspension of in-person classes even amidst pandemic-like situations.

It is well-established that international air traffic strongly influenced the rapid global transmission of SARS-CoV-2. The first detection of novel SARS-CoV-2 and its variants in most countries, including India, was usually due to inbound international travelers (Andrews *et al.* 2020; Li *et al.* 2023). Moreover, one study indicated that around 45% of initial infections in India were in districts with the busiest airports (Gupta *et al.* 2022). Kerala, a state of India with the largest number of international airports, has often reported the first case of several viral diseases or has witnessed several zoonotic disease outbreaks, including Nipah, Swine flu, SARS-CoV-2, and Monkeypox (Andrews *et al.* 2020; Yadav *et al.* 2022). Besides the USA and China, India is among the top three countries with the highest projected domestic and international air travelers. Currently, India has 17 international airports spread over 14 states (AAI 2024). Targeted wastewater analysis of WHO-listed priority organisms at important sentinel sites, for example, international airports, should be complemented with microbiome analysis using metagenomics and metatranscriptomics to detect the silent pathogen X, if any, providing early warning to health officials.

It is important to note that around 60% of human diseases have zoonotic origins and are often identified in humans before their actual animal source is known (Heymann David & Dixon 2013; Ahmed *et al.* 2024). If identified at the source before penetrating the animal–human interface, emerging zoonotic diseases could trigger immediate targeted response and emergency preparedness by public health agencies. Integrating wastewater surveillance with conventional livestock health surveillance methodologies could boost pandemic preparedness initiatives.

4.9. Develop a national WBE program

India needs to develop a national WBE program to strengthen public health monitoring efforts. Countries like Australia and the U.S. have been successful in setting up national-level wastewater surveillance programs for monitoring biomarkers in wastewater. A national WBE program can only be successful when each state and UT of India has its own state-wide wastewater monitoring program. The state/UT, in turn, can aggregate the WBE data from all district zones.

WBE studies should be conducted in partnership with the government and private laboratories, academic and medical research institutions. Existing laboratory facilities in each state, including those built during the COVID-19 pandemic, could be leveraged for the WBE pipeline. However, to make reliable comparisons of WBE-derived data, a standardized and optimized protocol must be established through inter-laboratory coordination and exchange of protocols. Inter-laboratory exercises in Europe have resulted in optimizing the analytical pipeline and improving WBE data quality (van Nuijs *et al.* 2018).

Alternative strategies must be envisioned if a WBE study experiences unexpected funding and supply constraints during the project execution. For instance, if reference standards are unaffordable, not immediately available, or regulatory controlled, tentative identification can be achieved using non-targeted analysis. Wastewater samples can also be sent overseas for analysis, provided that import/export permits are in place for cross-border transit and that sample integrity is maintained during the transit. Currently, several freight companies offer prioritized cold-chain sample delivery options from India to overseas.

WBE-derived data should feed into the proposed National Wastewater Informatics Centre and be publicly available on the proposed National WBE Dashboard. The proposed framework of the National WBE Program is shown in Figure 3. However, data ownership needs to be clearly delineated. The proposed framework may consider the ICMR as the nodal agency with data ownership and distribution rights. Additionally, ICMR, in coordination with the Indian Ministry of Health & Family Welfare, could oversee the national WBE program along with institutional partners at the state and district levels. ICMR could also take a lead role in the standardization of technical protocols and deciding reporting timelines applicable across all participating sites. The state public health departments could be made responsible for collecting data from individual districts, which in turn could be responsible for collecting data from local municipal bodies. Data aggregated at individual state levels should be relayed to the nodal agency, ensuring confidentiality. Data sharing in this multi-tiered setup should take place within a legal or regulatory framework, protecting communities' rights and ensuring safeguards against ethical violations. Capacity building is required in each step of the WBE protocol. WBE-focused seminars, workshops, training, and other outreach activities should be routinely conducted to disseminate and share scientific findings with field staff and encourage public participation. India would also need a few more dedicated research institutions along the lines of the Government of India's proposed National Institute for One Health at Nagpur, India, to focus on pandemic preparedness and disease prevention.

4.10. Ethical considerations

Although WBE studies are widely perceived to raise low ethical risk, caution is still needed while conducting studies and reporting the data (Hall *et al.* 2012). Researchers from Europe, the UK, and Australia have developed the ethical guidelines

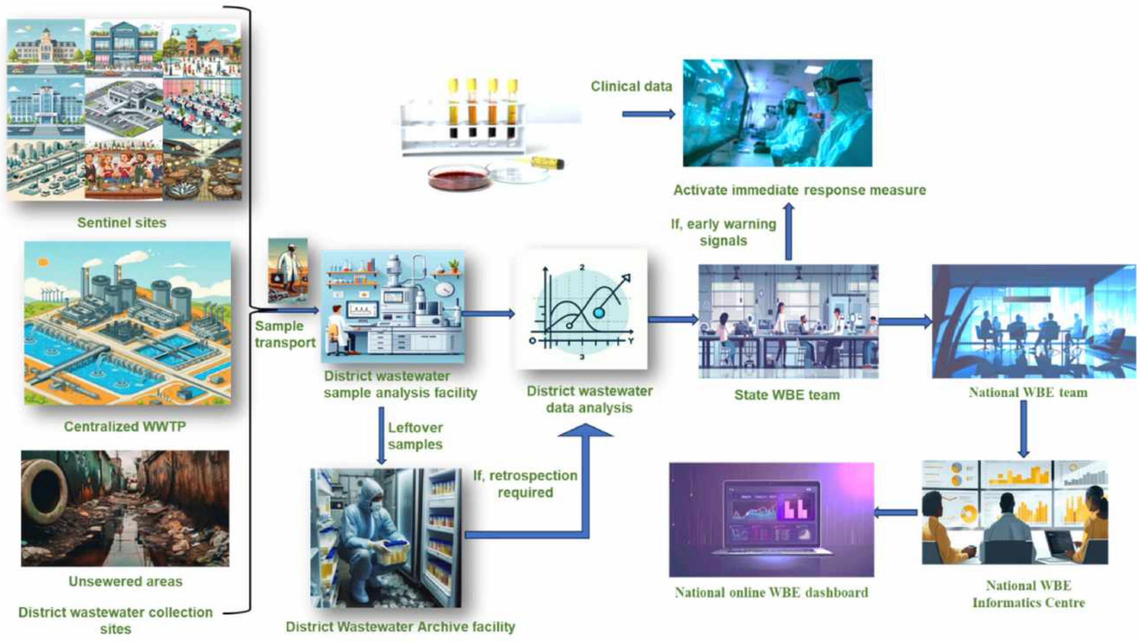


Figure 3 | Proposed national WBE framework.

for WBE and related fields (Prichard *et al.* 2023). India does not have any national ethical guidelines for WBE studies, which need immediate attention, especially given that the number of WBE studies has increased since the onset of the COVID-19 pandemic. The proposed ethical guideline for WBE could be integrated with the existing Indian Council for Medical Research (ICMR)'s ethical guidelines for public health research involving human participants. The high-resolution WBE study intended to be undertaken at the sub-catchment level with a small community, including prisons, housing apartments, schools, university campuses, hospitals, and offices, is more prone to ethical risk than a study done in a relatively large population at the level of a city or country. Consent of the municipal bodies and relevant authorities is needed before strategizing the sampling plan and publishing the name of the sampling location. WBE studies at sub-catchment levels are more prone to de-anonymization and data privacy breaches, potentially violating India's Digital Personal Data Protection Act 2023 (Ministry of Law and Justice, 2023). Additionally, sub-catchment WBE findings could contribute to disparities in real estate markets, health insurance premiums, and the socioeconomic structure of surveilled communities. There is a need to develop a policy to set up a minimum population threshold for high-resolution surveillance, thereby protecting communities' privacy and preventing potential data misuse.

Public health research findings attract extensive media attention and are ultimately conveyed to the general population. Community-level stigmatization and data misuse are other challenges to be addressed, particularly when reporting outcomes of substance use monitoring in vulnerable communities. WBE-derived data should be triangulated with secondary data, including survey outcomes, hospitalization records, and death rates, before communicating with communities to build and maintain public trust in this tool. Anonymizing locations where WBE is conducted is one approach to minimize population stigmatization. WBE data interpretation and communication beyond scientific publications require a thoughtful framing to mitigate potential ethical risks. Efforts should be made to encourage public participation at the early stage of the WBE framework and ensure them of the benefits of the study for their health and well-being. Community education and public campaigns are necessary tools to address any stigma attached to individuals involved in sample collection, specifically fecal sample collection.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

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