

WASTEWATER-BASED EPIDEMIOLOGY: ANALYTICAL METHODS FOR
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ABSTRACT

Background: Wastewater-based epidemiology (WBE) has evolved from a niche forensic tool into an essential, population-scale pillar of modern public health intelligence, offering non-invasive and objective insights into community health dynamics. **Objectives:** This review provides a comprehensive synthesis of contemporary literature on WBE, explicitly focusing on advanced analytical workflows for tracking community drug use, unintentional chemical exposures, epidemic viral pathogens, and antimicrobial resistance (AMR). **Methods:** Following a systematic bibliographic search across major scientific repositories (PubMed/Medline, Scopus, Web of Science, and Engineering Village), we evaluated recent advances in sample collection, pre-treatment, concentration, and quantification. Examined analytical platforms include liquid chromatography-tandem mass spectrometry (LC-MS/MS), environmental proteomics, reverse transcription-quantitative PCR (RT-qPCR), droplet digital PCR (ddPCR), next-generation sequencing (NGS) metaviromics, and field-deployable biosensors. **Results:** Findings demonstrate that rigorous control over pre-analytical variables—such as in-sewer transformation kinetics, biofilm degradation, and biomarker stability—is vital for accurate per capita back-calculation. Wastewater surveillance successfully circumvents the reactive bottlenecks of traditional clinical reporting, providing robust early-warning lead times ranging from 4 to 17 days for viral resurgences like influenza and SARS-CoV-2. Furthermore, urban sewer networks and hospital wastewater influents act as critical ecological hotspots, concentrating clinically vital resistance determinants (sul1/2, ermB/F, qnrS, mcr variants, and beta-lactamase genes) driven by therapeutic selection pressures and horizontal gene transfer. **Conclusions:** The convergence of advanced molecular detection, environmental proteomics, point-of-need biosensors, and artificial intelligence-driven predictive modeling is transforming WBE into a proactive early-warning architecture. Institutionalizing WBE within a unified One Health framework remains vital for safeguarding global health security and preempting future public health crises.

KEYWORDS: Wastewater-based epidemiology (WBE); Pathogen surveillance; Antimicrobial resistance (AMR); Environmental proteomics; Biosensors; Biomarker stability; Predictive modeling; One Health.

1. INTRODUCTION

Modern public health is continuously challenged by complex, population-wide phenomena, among which the escalating consumption of psychoactive substances and the global crisis of antimicrobial resistance (AMR) stand out as critical threats.^[2,5,12] Environmental chemical

pollution, shifting lifestyle behaviors, and biological selective pressures continue to undermine global health security and economic stability.^[2,6,12] While illicit and prescription drug abuse imposes an immense socio-economic burden on urban centers^[2,6], bacterial antimicrobial resistance accounts for millions of annual

deaths worldwide, threatening to render essential medical treatments ineffective.^[12]

Traditional epidemiological frameworks rely predominantly on clinical diagnostic testing, patient self-reporting, healthcare visits, and hospital admission registries.^[7,12,13] These conventional surveillance systems exhibit fundamental vulnerabilities; they are inherently reactive, labor-intensive, and subject to severe reporting and participation biases.^[7,12,13] Furthermore, they consistently fail to capture asymptomatic individuals, non-hospitalized carriers, or populations with limited healthcare access, particularly in resource-constrained regions where diagnostic infrastructure is easily overwhelmed during public health surges.^[3,7,12,13]

The Paradigm Shift to Wastewater-Based Epidemiology (WBE)

To bypass the diagnostic bottlenecks inherent in clinical reporting, Wastewater-Based Epidemiology (WBE) has risen as a transformative, non-invasive, and objective community health surveillance tool.^[2,3,11,15] WBE leverages the principle that human metabolic excretions, unabsorbed pharmaceutical residues, and microbial pathogen shedding are continuously deposited into municipal sewer networks.^[2,15] By pooling these biological inputs from entire connected communities, WBE offers an anonymous, population-wide perspective that operates entirely independently of individual healthcare-seeking behaviors or clinical testing capacities.^[2,7,13,15] While WBE gained worldwide visibility during the COVID-19 pandemic for tracking viral pathogens and emerging variants,^[3,4,7,13,15] its foundational methodologies are equally critical for tracking longitudinal lifestyle trends, chemical exposures, and the dissemination of antimicrobial resistance determinants.^[2,5,12]

Analytical Tracking of Community Drug Use

Originally conceptualized to monitor municipal wastewater for illicit drug residues^[2,6], WBE has matured into a robust, validated methodology for quantifying community-wide drug consumption and lifestyle patterns.^[2,5,6] Tracking specific human urinary biomarkers—such as benzoylecgonine for cocaine, ethyl sulfate for alcohol, and 1,7-dimethyluric acid for caffeine—allows researchers to back-calculate precise per capita consumption rates.^[2,5]

However, translating raw influent concentrations into accurate epidemiological metrics requires rigorous control over analytical variables.^[2,5] Compound stability during sewer transport, in-sewer biological transformation mediated by sewer biofilms, sorption onto suspended particulate matter, and human excretion kinetics introduce substantial analytical challenges.^[2,5] Consequently, selecting robust, human-specific biomarkers and establishing reliable population-normalization factors (such as acesulfame or

crAssphage) are paramount to mitigating estimation uncertainties and ensuring high data fidelity.^[2,5,6]

Expanding WBE to Antimicrobial Resistance (AMR) Surveillance

Beyond substance use tracking, WBE has expanded into monitoring antimicrobial resistance (AMR), addressing a vital One Health surveillance need.^[12,13] Urban sewer systems function as dynamic ecological interfaces where antibiotic-resistant bacteria (ARB), mobile genetic elements (MGEs), bacteriophages, and extracellular antibiotic resistance genes (ARGs) converge from diverse residential, agricultural, and industrial sources.^[12]

Hospital wastewater influents serve as prominent localized hotspots, discharging elevated loads of clinically critical resistance determinants—such as beta-lactamase genes, *sul1/2*, *ermB/F*, *qnrS*, and *mcr* variants—due to intense therapeutic selection pressures.^[12] Furthermore, residual antibiotic active pharmaceutical ingredients entering sewersheds drive horizontal gene transfer (HGT) and select for multidrug-resistant strains within sewer biofilms and activated sludge systems.^[5,12] Tracking these resistomes via WBE provides an unmasked view of environmental and clinical resistance reservoirs.^[12]

Technological Evolution: Analytical Methods and Smart Diagnostics

The analytical backbone driving WBE has evolved significantly, progressing from basic chromatographic screening to high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS), advanced PCR and droplet digital PCR (ddPCR) platforms, emerging optical and electrochemical biosensors, and environmental proteomics.^[2,9,10,11,12] These technological advancements enable both targeted and untargeted multi-chemical mining, allowing for the simultaneous tracking of trace drug metabolites and microbial resistance profiles.^[9,10]

Moreover, the integration of WBE with digital technologies—including the Internet of Things (IoT), artificial intelligence (AI), machine learning time-series forecasting, and cloud-fog computing—is steering the discipline toward smart diagnostics, transforming passive wastewater monitoring into a predictive, real-time public health early-warning system.^[11,15]

Review Scope and Objectives

Despite these major technical leaps, critical challenges persist regarding sample pre-concentration protocols, matrix interferences, standardization gaps, and data interpretation complexities.^[2,5,12,13,15] This review provides a comprehensive synthesis of the literature surrounding wastewater-based epidemiology, explicitly focusing on analytical methods for tracking community drug use and antimicrobial resistance.^[2,5,11,12] By evaluating compound stability, biomarker selection criteria, source-specific discharge dynamics, and

advanced analytical workflows, this work aims to establish an authoritative reference framework for optimizing WBE as a cornerstone of modern public health and environmental surveillance.^[2,5,11,12]

2. REVIEW OF LITERATURE

Wastewater-based epidemiology (WBE) has evolved from a niche analytical approach into a transformative, population-wide community health surveillance paradigm. Historically utilized for monitoring illicit drug consumption and tracking polio eradication efforts, WBE experienced an unprecedented global expansion during the COVID-19 pandemic.^[3,4,15] Data-driven bibliometric and knowledge graph analyses reveal that WBE research has transitioned into a highly collaborative, multidisciplinary field bridging environmental chemistry, molecular biology, public health, and data science.^[6] By analyzing human metabolic excretions, pharmaceutical residues, and microbial shedding pooled within municipal sewer networks, WBE provides an objective, anonymous snapshot of public health status entirely independent of individual healthcare-seeking behaviors or clinical testing capacities.^[2,7,13,15]

Analytical Methodologies and Technological Advancements

The analytical backbone of WBE has progressed significantly, moving from basic chromatographic screening to high-resolution mass spectrometry (LC-MS/MS), advanced PCR platforms, and innovative biosensors.^[9,10,15]

- **Environmental Proteomics & Immunoproteomics:** Recent advancements have expanded WBE beyond small molecules to encompass large macromolecules such as proteins, host immunoglobulins (IgG, IgA), cytokines, and pathogen-specific antigens. Immunoproteomics enables the assessment of community-level immune status, infection prevalence, and vaccination coverage.^[10]
- **Biosensing Technologies:** Near-real-time and on-site surveillance have been bolstered by electrochemical and optical biosensors equipped with biological recognition elements (antibodies, aptamers, nanobodies). These devices achieve high sensitivity in complex matrices when paired with robust sample cleanup and antifouling strategies.^[9,11]
- **Biomarker Stability:** A critical factor in quantitative WBE is compound stability during in-sewer transport and sample storage. While many cardiovascular drugs, antidiabetic agents (e.g., metformin), and specific illicit drug metabolites (e.g., benzoylecgonine, THC-COOH) exhibit high stability, others require careful temperature control and optimized pretreatment protocols to prevent degradation.^[5]

Infectious Disease and Pandemic Surveillance

WBE has established itself as an indispensable early-warning system for tracking infectious disease outbreaks and community transmission dynamics.^[7,15]

- **Viral Pathogen Tracking:** Beyond SARS-CoV-2, wastewater surveillance has successfully targeted seasonal influenza viruses, respiratory syncytial virus (RSV), enteroviruses, norovirus, and emerging threats such as the mpox virus. Systematic reviews on influenza and small-scale WBE demonstrate that wastewater viral loads correlate strongly with clinical case counts and hospital admissions, often providing crucial lead times ahead of clinical reporting.^[7,13,14]
- **Small-Scale and Building-Level Surveillance:** While centralized wastewater treatment plant (WWTP) monitoring captures city-wide trends, small-scale WBE operating at the neighborhood, school, or healthcare facility level offers fine-grained spatial resolution. Small-scale monitoring pinpoints local transmission clusters, identifies institutional outbreaks, and enables targeted public health interventions.^[13]
- **Global One Health Integration:** International frameworks such as the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON) emphasize integrating wastewater monitoring into One Health initiatives. This holistic approach connects human, animal, and environmental health to detect zoonotic spillovers, monitor animal-reservoir pathogens (e.g., avian influenza A/H5N1), and enhance global pandemic preparedness.^[8]

Expanding Frontiers: Antimicrobial Resistance, Chemical Exposures, and Chronic Disease Biomarkers

The scope of WBE continues to broaden into critical areas beyond traditional pathogen and drug monitoring:

- **Antimicrobial Resistance (AMR):** Urban sewer networks serve as dynamic ecological interfaces where antibiotic-resistant bacteria (ARB), mobile genetic elements (MGEs), and antibiotic resistance genes (ARGs) converge. Hospital wastewater influents act as prominent localized hotspots discharging elevated loads of clinically critical resistance determinants (beta-lactamase genes, *sul1/2*, *ermB/F*, *qnrS*, *mcr* variants) driven by intense therapeutic selection pressures.^[12]
- **Unintentional Chemical Exposure:** Unlike licit or illicit drugs, human exposure to environmental pollutants—such as per- and polyfluoroalkyl substances (PFAS), organophosphorus flame retardants, phthalates, bisphenols, parabens, and pesticides—occurs unintentionally. Tracking exposure biomarkers in wastewater and sewage sludge helps evaluate community-level xenobiotic

burdens and supports human biomonitoring (HBM) integration.^[2]

- **Non-Communicable Diseases and Emerging Biomarkers:** Early exploratory frameworks are investigating the feasibility of tracking non-communicable health conditions through WBE, such as identifying urinary biomarkers for Alzheimer's disease (e.g., amyloid- β , tau, AD7C-NTP, long non-coding RNAs, and oxidative stress markers) in municipal sewage.^[1]

3. METHODOLOGY

A comprehensive literature search was conducted to identify peer-reviewed studies, systematic reviews, and technical evaluations concerning wastewater-based epidemiology (WBE), analytical workflows, community drug consumption, and antimicrobial resistance (AMR) tracking.

- **Information Sources and Search Strategy:** A structured bibliographic search was executed across major scientific repositories, including PubMed/Medline, Scopus, Web of Science, and Engineering Village. The search strings utilized combinations of keywords and Boolean operators designed to capture relevant literature without geographical or temporal restrictions up to 2025. Primary search terms included: ("wastewater-based epidemiology" OR "sewage surveillance" OR "wastewater surveillance") AND ("analytical methods" OR "LC-MS/MS" OR "biomarker stability" OR "environmental proteomics" OR "biosensors") AND ("community drug use" OR "illicit substances" OR "antimicrobial resistance" OR "AMR" OR "resistance genes" OR "viral pathogen tracking").
- **Inclusion and Exclusion Criteria:** Literature selection was governed by strict eligibility parameters. Studies were included if they met the following criteria:
 1. Peer-reviewed original research articles, bibliometric evaluations, or comprehensive reviews focusing on wastewater surveillance architectures, sampling protocols, concentration methods, and analytical quantification platforms.
 2. Investigations addressing community-wide drug consumption, lifestyle biomarkers, chemical exposure metrics, or the dissemination of antimicrobial resistance determinants and mobile genetic elements within urban sewer networks.
 3. Articles published in the English language with accessible full-text data.Non-peer-reviewed commentaries, editorials, conference abstracts lacking methodological detail, and studies outside the scope of environmental or public health surveillance were systematically excluded.
- **Study Selection and Data Extraction:** Following the removal of duplicate records retrieved across

multiple electronic databases, titles and abstracts were screened for thematic relevance. Full texts of shortlisted articles were thoroughly evaluated to extract key insights regarding pre-analytical variables, in-sewer transformation kinetics, multi-omics integration, and public health applications.

4. RESULTS

Bibliometric Evolution and Research Landscape of Wastewater-Based Epidemiology

Bibliometric evaluations and data-driven text-mining analyses of the global wastewater-based epidemiology (WBE) literature reveal an exponentially expanding research field, characterized by strong multidisciplinary collaboration across environmental science, analytical chemistry, infectious disease medicine, and microbiology.^[6] While publication output remained modest prior to 2013, the field experienced a rapid upward trajectory, culminating in an unprecedented surge in output post-2020 driven by the global response to the COVID-19 pandemic.^[6] Geographically, research leadership and academic influence are heavily concentrated in the United States, Australia, China, and Spain.^[6] Keyword trend analyses demonstrate a distinct thematic shift from early primary focal points on illicit drugs and standard pharmaceuticals toward new psychoactive substances (NPS), biomarker stability dynamics, immunoproteomics, and real-time viral surveillance.^[6]

Analytical Workflows: Sampling, Sample Pre-Treatment, and Detection Platforms

The integrity of WBE outcomes depends on standardized analytical workflows encompassing strategic sampling, pre-treatment, concentration, and quantification.^[3,15]

- **Sampling Strategies:** Sampling configurations range from centralized municipal wastewater treatment plant (WWTP) inlets to high-resolution neighborhood (sub-sewershed) and building-level (e.g., hospitals, schools, university dormitories) collection points.^[13,15] Sampling modalities include grab sampling, 24-hour time- or flow-proportional composite sampling, and low-cost passive samplers (such as Moore swabs).^[3,13]
- **Pre-Treatment and Concentration:** Because target biomarkers and viral titers in wastewater are frequently present at trace levels, primary pre-treatment (such as initial coarse filtration, grit removal, or centrifugation) is required.^[3,15] Secondary concentration methodologies include polyethylene glycol (PEG) precipitation, ultrafiltration, electronegative membrane adsorption-elution, and ultracentrifugation.^[3,7,15] Notably, comparative evaluations for respiratory and enveloped viruses demonstrate that focusing on the solid fraction (primary sludge, settled solids, or pelleted particulate matter) frequently outperforms liquid supernatant fractions due to the hydrophobic partitioning and adherence of viral lipid envelopes to suspended solids.^[14]

- **Detection and Quantification Platforms:** Laboratory analysis relies predominantly on molecular techniques, including quantitative reverse transcription PCR (RT-qPCR), droplet digital PCR (ddPCR) for absolute quantification, targeted amplicon sequencing, and next-generation sequencing (NGS) metaviromics.^[3,7,14,15] To account for fluctuations in population size, catchment dilution, and human excretion variations, data normalization is frequently applied using faecal indicators such as Pepper Mild Mottle Virus (PMMoV), crAssphage, or hydraulic flow parameters.^[3,7,14,15]

Community Drug Consumption, Lifestyle Markers, and Unintentional Chemical Exposures

WBE has achieved a high degree of methodological maturity in tracking community-wide consumption of licit and illicit substances as well as unintentional chemical exposures.^[2,5]

- **Illicit and Licit Substances:** Estimating per capita consumption requires tracking stable human urinary excretion biomarkers, such as benzoylecgonine for cocaine, 1,7-dimethyluric acid for caffeine, and ethyl sulfate for alcohol.^[2,5]
- **Compound Stability:** Compound stability during sewer transport is a primary determinant of back-calculation accuracy.^[5] While many illicit drugs and pharmaceutical classes (such as cardiovascular drugs and the antidiabetic agent metformin) exhibit high sewer stability, parent compounds like cocaine, heroin, and THC degrade rapidly and necessitate monitoring via stable human metabolites (benzoylecgonine, morphine, and THC-COOH, respectively).^[5]
- **Unintentional Chemical Exposures:** Beyond substance abuse, WBE effectively captures population-wide exposure to harmful organic contaminants, including phthalates, pesticides, flame retardants, bisphenols, per- and polyfluoroalkyl substances (PFAS), parabens, and UV filters.^[2] However, in-sewer biological transformations—such as the microbial hydrolysis of parent phthalates into monoester metabolites—introduce significant analytical variables that must be accounted for to avoid overestimating human exposure.^[2]

Pathogen Surveillance, Viral Dynamics, and Pandemic Preparedness

Wastewater surveillance has been extensively validated for tracking enteric pathogens (poliovirus, norovirus, hepatitis viruses, rotavirus) and respiratory viral agents.^[7,14]

- **Influenza Surveillance:** Systematic evaluations of wastewater-based influenza epidemiology confirm that influenza A and B viruses are consistently detectable across diverse geographical settings.^[14] Studies report moderate-to-strong positive correlations between environmental viral loads and clinical indicators (such as emergency department

visits, test positivity rates, and hospital admissions).^[14] Furthermore, time-shift analyses indicate that wastewater detection frequently precedes clinical reporting, providing an early-warning lead time ranging from 4 to 17 days.^[14]

- **Pandemic Potential and Zoonotic Spillover:** Global frameworks highlight WBE as a vital sentinel network for identifying zoonotic spillovers and high-consequence pathogens (e.g., mpox, arboviruses like dengue and Zika, and avian influenza A/H5N1).^[7,8] Genomic sequencing and amplicon analysis enable researchers to distinguish between animal reservoir contamination and sustained human-to-human transmission, as well as track the emergence of novel viral variants of concern (VOCs).^[7,8,14]

Surveillance of Antimicrobial Resistance (AMR) in Wastewater Environments

Urban sewer networks function as major ecological reservoirs and active mixing vessels for antimicrobial resistance (AMR), establishing WBE as an essential One Health surveillance strategy.^[12]

- **Resistome Composition:** Prevalent antibiotic-resistant bacterial (ARB) species identified in municipal influents include *Escherichia coli*, *Pseudomonas* spp., and *Enterococcus* spp.^[12] Concurrently, prominent antibiotic resistance genes (ARGs) detected across global studies encompass beta-lactamase genes (bla variants), sulfonamide resistance genes (sul1/2), macrolide resistance determinants (ermB/F), quinolone resistance genes (qnrS), colistin resistance genes (mcr variants), and tetracycline resistance genes (tetM).^[12]
- **Hospital Discharge Dynamics:** Comparative analyses consistently demonstrate that hospital wastewater influents serve as prominent localized hotspots, exhibiting significantly higher concentrations of clinically critical resistance determinants and mobile genetic elements (MGEs) compared to general municipal influents.^[12] This differential reflects heavy therapeutic selection pressures within healthcare settings.^[12] Furthermore, residual active pharmaceutical ingredients (such as azithromycin, ciprofloxacin, and clarithromycin) entering sewersheds drive horizontal gene transfer (HGT) and select for multidrug-resistant strains within sewer biofilms and activated sludge systems.^[12]

Technological Innovations: Proteomics, Biosensors, and Smart Diagnostics

The technical architecture of WBE is undergoing a rapid paradigm shift driven by cutting-edge analytical innovations.^[9,10,11,15]

- **Environmental Proteomics and Immunoproteomics:** Mass spectrometry-based proteomics (LC-MS/MS) and immunoproteomic assays facilitate the deep profiling of wastewater matrices, enabling the detection of human host

proteins (such as uromodulin, S100A8, and defensins), mucosal immunoglobulins (IgG, IgA), inflammatory markers (C-reactive protein), and pathogen-specific antigens.^[10] This immunological dimension provides direct insight into community immune status and infection prevalence.^[10]

- **Point-of-Need Biosensors:** To mitigate the high costs, specialized infrastructure, and operational delays associated with centralized laboratory RT-qPCR, diverse biosensing platforms—including paper-based microfluidics, electrochemical aptasensors, and nanomaterial-based devices—have been developed to offer rapid, cost-effective, and field-deployable detection.^[9,11]
- **Smart Diagnostics and Predictive Modeling:** The integration of WBE datasets with digital technologies—including the Internet of Things (IoT), cloud-fog computing, and artificial intelligence/machine learning time-series forecasting models (e.g., Artificial Neural Networks and Random Forests)—is steering the discipline toward smart diagnostics, transforming passive wastewater monitoring into a predictive, real-time public health early-warning system.^[11,15]

5. DISCUSSION

The synthesis of contemporary literature underscores that Wastewater-Based Epidemiology (WBE) has evolved from a niche forensic tool into an essential, population-scale pillar of modern public health intelligence.^[3,7,12] Moving far beyond its early applications in tracking illicit substance consumption^[6], WBE now offers a dynamic, non-invasive window into community health status, chemical exposure, viral transmission dynamics, and the global spread of antimicrobial resistance (AMR).^[2,3,7,12] However, translating raw wastewater measurements into actionable public health insights requires navigating complex analytical, biochemical, and methodological challenges.^[2,5,15]

- **Methodological Complexities and Biomarker Stability:** A central theme emerging from the literature is the profound influence of pre-analytical and analytical variables on WBE data fidelity.^[2,5,15] As highlighted by biomarker stability studies, the fate of target compounds during in-sewer transport—dictated by temperature, hydraulic retention time, pH, sorption onto suspended particulate matter, and biological degradation by sewer biofilms—can introduce substantial uncertainty.^[2,5] While many licit and illicit pharmaceutical classes exhibit high sewer stability, labile parent compounds (such as cocaine, heroin, and THC) degrade rapidly and require monitoring through stable human metabolites like benzoylecgonine and THC-COOH.^[5] Similarly, evaluating unintentional chemical exposures (e.g., phthalates or bisphenols) is complicated by non-human point sources, industrial discharges, and in-

sewer hydrolysis, which can lead to significant overestimations of human exposure if uncorrected.^[2]

- **Bridging Clinical Surveillance Gaps via Multi-Pathogen and AMR Tracking:** Traditional clinical surveillance is inherently reactive, labor-intensive, and prone to severe underreporting due to healthcare access disparities and high rates of asymptomatic carriage.^[3,7,12] WBE successfully circumvents these bottlenecks by capturing pooled community shedding independently of healthcare-seeking behavior.^[3,7,13] Wastewater surveillance of SARS-CoV-2 and seasonal influenza viruses exhibits strong positive correlations with clinical indicators (emergency department visits, test positivity rates, and hospital admissions).^[3,14] Crucially, time-shift analyses reveal that wastewater detection frequently precedes clinical reporting, providing an indispensable early-warning lead time ranging from 4 to 17 days.^[14] Concurrently, WBE provides critical insights into the environmental reservoir of antimicrobial resistance (AMR).^[12] Urban sewer networks act as ecological mixing vessels where antibiotic-resistant bacteria (*Escherichia coli*, *Pseudomonas* spp., *Enterococcus* spp.) and mobile genetic elements converge.^[12] Hospital wastewater influents function as prominent localized hotspots, discharging significantly higher concentrations of clinically critical resistance determinants (such as beta-lactamase genes, *sul1/2*, *ermB/F*, *qnrS*, and *mcr* variants) due to intense therapeutic selection pressures.^[12] Residual active pharmaceutical ingredients entering sewersheds further drive horizontal gene transfer (HGT) within sewer biofilms, underscoring the necessity of integrating WBE into a broader One Health surveillance framework.^[5,12]
- **The Digital Transformation: Toward Smart Diagnostics and Predictive Modeling:** The technical architecture of WBE is undergoing a rapid paradigm shift driven by technological innovations.^[9,10,11,15] Environmental proteomics and immunoproteomics bridge environmental surveillance with clinical immunology by detecting human host proteins (uromodulin, S100A8, defensins), mucosal immunoglobulins (IgG, IgA), and pathogen-specific antigens.^[10] Simultaneously, the development of rapid, field-deployable biosensing platforms (paper-based microfluidics, electrochemical aptasensors) mitigates the logistical bottlenecks of centralized laboratory RT-qPCR.^[9,11] Crucially, the integration of WBE datasets with digital technologies—including the Internet of Things (IoT), cloud-fog computing architectures, and artificial intelligence/machine learning time-series forecasting models (Artificial Neural Networks and Random Forests)—is steering the discipline toward smart diagnostics, transforming passive wastewater monitoring into a predictive, real-time public health early-warning system.^[11,15]

6. CONCLUSION

Wastewater-Based Epidemiology has firmly established itself as an indispensable, non-invasive, and objective pillar of modern public health and environmental surveillance.^[3,7,12,15] By bridging the blind spots of conventional clinical reporting, WBE delivers population-wide intelligence on community drug consumption trends, chemical exposures, viral dynamics, and antimicrobial resistance profiles.^[2,3,7,12] While methodological challenges—such as matrix interference, sewer degradation kinetics, and standardization gaps—remain, the convergence of advanced molecular detection, environmental proteomics, point-of-need biosensors, and machine learning predictive modeling has transformed WBE from a passive monitoring tool into a proactive, real-time early-warning architecture.^[2,5,9,10,11,12,15] Ultimately, institutionalizing WBE within a unified One Health framework is essential for safeguarding global health security and preempting future public health crises.^[8,12,15]

FUTURE SCOPE

To fully realize the operational potential of WBE and transition wastewater monitoring into a routine, globally standardized public health asset, future research and development must focus on the following key domains:

- **Multi-Omics Integration:** Advancing comprehensive multi-omics pipelines that seamlessly couple metagenomics, environmental proteomics, metabolomics, and transcriptomics to provide a holistic assessment of community health, metabolic burdens, and microbial resistomes.^[10,15]
- **Global Standardization and Harmonization:** Establishing internationally unified protocols for wastewater sampling configurations, sample pre-concentration, quality control procedures, and data reporting metrics to ensure cross-study comparability and eliminate geographical surveillance biases.^[8,12,15]
- **Equitable Implementation in LMICs:** Overcoming economic and infrastructural barriers in low- and middle-income countries (LMICs) by developing cost-effective, decentralized, and field-deployable tools (such as low-cost passive samplers and robust biosensors) tailored for resource-constrained settings.^[8,9,13]
- **Small-Scale and High-Resolution Mapping:** Expanding building-level (hospitals, schools) and neighborhood sub-sewershed surveillance networks to resolve fine-grained intra-urban epidemiological variations and target localized transmission hotspots.^[13]
- **Ethical and Privacy Governance:** Formulating robust legal and ethical frameworks to govern small-scale and building-level wastewater surveillance, ensuring strict data anonymization, protecting community privacy, and preventing social stigmatization.^[8]

- **Broadening Biomarker Panels:** Extending WBE applications beyond infectious diseases and substance use to monitor non-communicable disease indicators (e.g., urinary biomarkers for neurodegenerative disorders), nutritional markers, and chronic environmental toxin exposures.^[1,2]

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