

Review

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Review

# A Review of Gastrointestinal and Respiratory Pathogen Detection in Wastewater: Implications for Early Warning and Public Health Surveillance

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## Abstract

In Africa, the load of diarrheal and respiratory diseases is amplified by limited surveillance capacity, diagnostic limitations, and socioeconomic inequalities. In rapidly urbanizing settings such as Kigali (Rwanda), integrating wastewater-based epidemiology (WBE) into existing surveillance systems gives a promising strategy for generating real-time epidemiological intelligence, identifying community-level hotspots, and addressing gaps in traditional reporting systems. Gastrointestinal and respiratory infections remain major causes of morbidity and mortality globally, mainly in low- and middle-income countries (LMICs), where traditional clinical surveillance systems frequently underreport true disease burden. This review synthesizes present evidence on the detection of gastrointestinal and respiratory pathogens in wastewater and evaluates the utility of WBE for early warning and public health action. A narrative review approach was used to identify peer-reviewed literature, global health reports, and surveillance studies focusing on wastewater detection of gastrointestinal and respiratory pathogens. Databases including PubMed, Scopus, and Google Scholar were searched for studies published between 2000–2024. The search yielded 1,247 records, of which 312 duplicates were removed. After title/abstract screening, 228 full texts were assessed for eligibility. Ultimately, 96 studies met the inclusion criteria and were included in the final review. Reasons for exclusion included: lack of pathogen data (n=58), industrial focus (n=27), or insufficient methodological detail (n=47). WBE provides a non-invasive, cost-effective approach for monitoring symptomatic and asymptomatic infections. Challenges involve variability in sampling, environmental factors affecting viral decay, and differences in laboratory workflows. WBE is a powerful complement to traditional infectious disease surveillance, suggesting early warning capabilities, population-level coverage, and real-time insights into pathogen circulation. Integrating WBE into surveillance programs especially in LMICs such as Rwanda can significantly strengthen epidemic preparedness, guide resource allocation, and improve outbreak response. Sustained investment in laboratory capacity, standardized protocols, and multisector collaboration is essential to fully leverage WBE for public health protection.

**Keywords:** wastewater-based epidemiology; gastrointestinal and respiratory pathogens; early warning; public health surveillance

## 1. Introduction

Wastewater-based epidemiology (WBE) has emerged as a powerful, non-invasive tool for monitoring infectious diseases at the community level, suggesting timely and cost-effective insights

into pathogen circulation [1–3] Globally, gastrointestinal and respiratory infections remain among the most meaningful public health threats, extremely affecting populations in low- and middle-income countries (LMICs) due to prompt rapid population growth, urbanization, climate change, stressed sanitation systems, and rising antimicrobial resistance [4–7]. These factors contribute to the spread and re-emergence of enteric and respiratory pathogens, generating an urgent need for advanced surveillance systems capable of detecting both symptomatic and asymptomatic infections. Traditional clinical surveillance reliant on healthcare-seeking performance, laboratory diagnostics, and broadcasting systems captures only a portion of true cases, particularly in resource-limited situations where diagnostic coverage is uneven and health-seeking patterns vary widely [8–10]. Throughout the COVID-19 pandemic, adjournments in detecting community transmission and reliance on clinical testing critically damaged response efforts and intensified transmission in many regions [11–13]. These limits have led to increasing global interest in environmental surveillance approaches, particularly WBE, as complementary tools for considerate pathogen transmission dynamics and strengthening epidemic preparedness such as in Rwanda. WBE includes analyzing sewage for genetic material or biomarkers shed by infected individuals, allowing the detection of both symptomatic and asymptomatic infections at a population level [1,3,14]. This approach has validated strong performance in tracking a wide range of pathogens, including SARS-CoV-2, norovirus, rotavirus, hepatitis A virus, *Campylobacter* spp., *Salmonella* spp., and protozoan parasites such as *Giardia* and *Cryptosporidium* [15–20] to mention but a few. Numerous studies have exhibited strong correlations between pathogen concentrations in wastewater and clinical case trends, with wastewater signals often preceding clinical waves by several days to weeks highlighting the value of WBE as an early warning system [12,17,21–23]. Gastrointestinal pathogens including *Escherichia coli*, *Salmonella*, *Vibrio cholerae*, norovirus, rotavirus, and hepatitis A virus remain major contributors to global morbidity and mortality, mostly among children under five in LMICs [6,18,19,24]. In addition, Also, respiratory pathogens, especially SARS-CoV-2 and influenza viruses, have been identified in wastewater, supporting the use of WBE for respiratory disease surveillance [12,22,25]. Wastewater monitoring consequently offers a critical opportunity to notice outbreaks, display endemic disease patterns, and value the effectiveness of public health interventions such as vaccination programs and increases in water and sanitation infrastructure [17,24,26]. In Africa, the load of diarrheal and respiratory diseases is amplified by limited surveillance capacity, diagnostic limitations, and socioeconomic inequalities [7,27,28]. In rapidly urbanizing settings such as Kigali (Rwanda), integrating WBE into existing surveillance systems gives a promising strategy for generating real-time epidemiological intelligence, identifying community-level hotspots, and addressing gaps in traditional reporting systems [28–30]. Evidence from the COVID-19 pandemic revealed that WBE can detect emerging variants, identify resurgences even when clinical testing failures, and support data-driven decision-making in resource-limited environments [12,21,30–32]. Given these advantages, understanding in what way WBE can be leveraged for multi-pathogen detection in Kigali and similar urban centers is vital for strengthening public health preparedness and response. This review synthesizes up-to-date evidence on the finding of gastrointestinal and respiratory pathogens in wastewater, evaluates relationships between wastewater pathogen strengths and clinical case patterns, and emphasizes the role of WBE as an early warning tool. Furthermore, we discuss methodological considerations, challenges, and opportunities for integrating WBE into national disease surveillance frameworks in Rwanda and within Africa.

## 2. Materials and Methods

### 2.1. Why the Africa Context Matters?

In Africa with over 50 countries and rapidly expanding cities, The African context, particularly urbanizing cities such as Kigali is critically important for advancing wastewater-based epidemiology (WBE). Many African countries experience an uneven burden of infectious diseases and frequent outbreaks, while investigation systems often face diagnostic delays due to reduced laboratory

capacity and under-reporting of clinical cases [33,34]. Fast population growth, dense settlements, and uneven health infrastructure additional increase the risk of silent transmission, making early detection essential [35]. Kigali, Rwanda's capital and largest city with about two million population (2022 Rwandan Census) exemplifies a rapidly developing urban environment where expanding wastewater networks and high population mobility generate both challenges and opportunities for implementing WBE as a cost-effective surveillance tool [36,37]. Besides providing population-level, near-real-time pathogen insights, WBE can strengthen early-warning systems, complement traditional surveillance, and support more equitable outbreak readiness across African settings [14,38,39]. This systematic review was led following PRISMA principles to safeguard transparency and reproducibility. Five electronic databases were searched: PubMed/MEDLINE, Scopus, Web of Science, and the African Journals Online (AJOL) platform. The search covered studies published between January 2000 and October 2024 October 2025. A comprehensive Boolean search strategy combining Medical Subject Headings (MeSH) and free-text terms was applied. The core search series included combinations of: wastewater-based epidemiology, wastewater surveillance, environmental surveillance, pathogen detection, Africa, Rwanda, Kigali, SARS-CoV-2, enteric pathogens, AMR, virus detection, and public health surveillance [14,16,38]. This assessment aims to synthesize current suggestion on wastewater-based epidemiology for early pathogen detection, with emphasis stress on its applicability in African frameworks. Latest studies reveal that WBE can reliably detect viral, bacterial, and antimicrobial-resistant pathogens and bring actionable public-health insights before clinical data become available [14,38–40]. Nonetheless, research coverage across Africa remains limited, despite demonstrated feasibility in countries such as South Africa, Nigeria, Rwanda, and Senegal [36,41–43]. In consolidating global and Africa-specific results, this review identifies methodological advances, data gaps, and integration opportunities for strengthening public-health surveillance. Eventually, it highlights the potential for WBE to serve as a scalable and sustainable early-warning system capable of refining disease preparedness in resource-limited sets such as Kigali.

## 2.2. Inclusion Criteria

Studies were considered eligible for inclusion in this review if they reported either primary or secondary data on the detection of pathogens in wastewater. Eligible studies were essential to present microbiological, genomic, molecular or epidemiological outcomes significant to wastewater-based epidemiology (WBE). In addition, studies conducted in African settings were selected, as well as those specifying methodological understandings applicable to low-resource or resource-limited frameworks. Peer-reviewed articles, preprints, technical reports, and governmental or institutional surveillance documents published in either English or French were incorporated to ensure comprehensive coverage of both scientific and policy-oriented evidence.

## 2.3. Exclusion Criteria

Studies were excluded from the review if they dedicated exclusively on industrial discharge or environmental monitoring without direct relevance to public health. Publications lacking empirical laboratory records or epidemiological evidence were also excluded, as were studies that did not report specific pathogen finding outcomes in wastewater. Moreover, duplicate records, editorials, opinion pieces, interpretations, and conference abstracts without full methodological descriptions were rejected to preserve methodological rigor and data reliability.

## 2.4. Screening Process

All recovered records were imported into Zotero for de-duplication, after which two independent reviewers selected titles and abstracts. Full texts of potentially eligible studies occurred then assessed for inclusion. Any differences were resolved through consensus or adjudication by a third reviewer. Data extraction focused on key study types and outcomes, including the country,

study setting, the specific pathogens targeted, the sampling and analytical methods employed. Extracted outcomes involved pathogen detection rates, assessable measurements, and any reported relationships between wastewater signals and corresponding clinical or epidemiological data. Furthermore, information on the public-health applications of wastewater-based epidemiology, such as early warning, surveillance integration, or outbreak response, was methodically collected.

### *2.5. Technical Workflow Overview*

This section provides a high-level overview of the biological and analytical workflows commonly used in wastewater-based epidemiology studies included in this review.

### *2.6. Sampling Workflow*

Most studies conducted in Africa and generally employed one of three primary wastewater-sampling strategies. Grab sampling was usually used, mostly at influent points of wastewater treatment plants or at sewer junctions, due to its effective simplicity. Composite sampling, frequently implemented using automated samplers, integrated flow-proportional or time-proportional samples over a 24-hour period to better apprehend temporal variability in pathogen shedding. Passive sampling approaches, involving Moore swabs and other absorbent materials, have gained increasing responsiveness, especially in low-resource settings such as Rwanda, where they offer a cost-effective and logistically feasible alternative while maintaining analytical sensitivity [36,44,45]. Sample preservation normally involved cooling at 4 °C during transport, with laboratory processing performed within 6–24 hours to minimize nucleic acid degradation and ensure data quality.

### *2.7. Concentration and Extraction Workflow*

Viral and bacterial targets in wastewater samples were concentrated prior to analysis exploiting a range of conventional methods. Normally applied approaches involved polyethylene glycol (PEG) precipitation, ultrafiltration, electronegative membrane adsorption–elution techniques, and flocculation-based protocols. The concentration method varied according to target pathogen type, available laboratory infrastructure, sample matrix and resource settings, with each methodology offer distinct advantages in terms of recovery efficiency, scalability, and operational feasibility. RNA and DNA extractions were performed using commercial kits (e.g., Qiagen, Promega Maxwell, ThermoFisher) or phenol-chloroform-based protocols, depending on laboratory capacity. Internal process controls such as murine hepatitis virus (MHV), pepper mild mottle virus (PMMoV), and MS2 bacteriophage were often used to gauge recovery efficiency and normalization [40,46].

### *2.8. Biological and Molecular Detection Workflow*

Following nucleic acid extraction, pathogen detection and quantification were primarily performed by reverse transcription quantitative PCR (RT-qPCR) or quantitative PCR (qPCR) for viral and bacterial targets. Droplet digital PCR (ddPCR) was also used in several studies to reach higher analytical sensitivity and enhanced absolute quantification, particularly for low-abundance targets. In addition, amplicon-based or whole-genome sequencing approaches were used to sustain variant tracking, antimicrobial resistance profiling, and phylogenetic inference. To increase data quality and comparability across sampling sites and time facts, normalization and quality control strategies were applied, including the use of fecal indicators such as pepper mild mottle virus (PMMoV) and crAssphage, as well as adjustments based on wastewater flow rates [40,79]. This workflow reflects the methodologies used both globally and in emerging African studies, including Rwanda's SARS-CoV-2 wastewater surveillance pilot in Kigali, which demonstrated strong concordance between wastewater viral concentrations and reported clinical trends [4,36].

## **3. Results**

### *3.1. Prisma Layout Flow Summary*

The search yielded 1,247 records, of which 312 duplicates were removed. After title/abstract screening, 228 full texts were assessed for eligibility. Ultimately, 96 studies met the inclusion criteria and were included in the final review. Reasons for exclusion included: lack of pathogen data (n=58), industrial focus (n=27), or insufficient methodological detail (n=47).

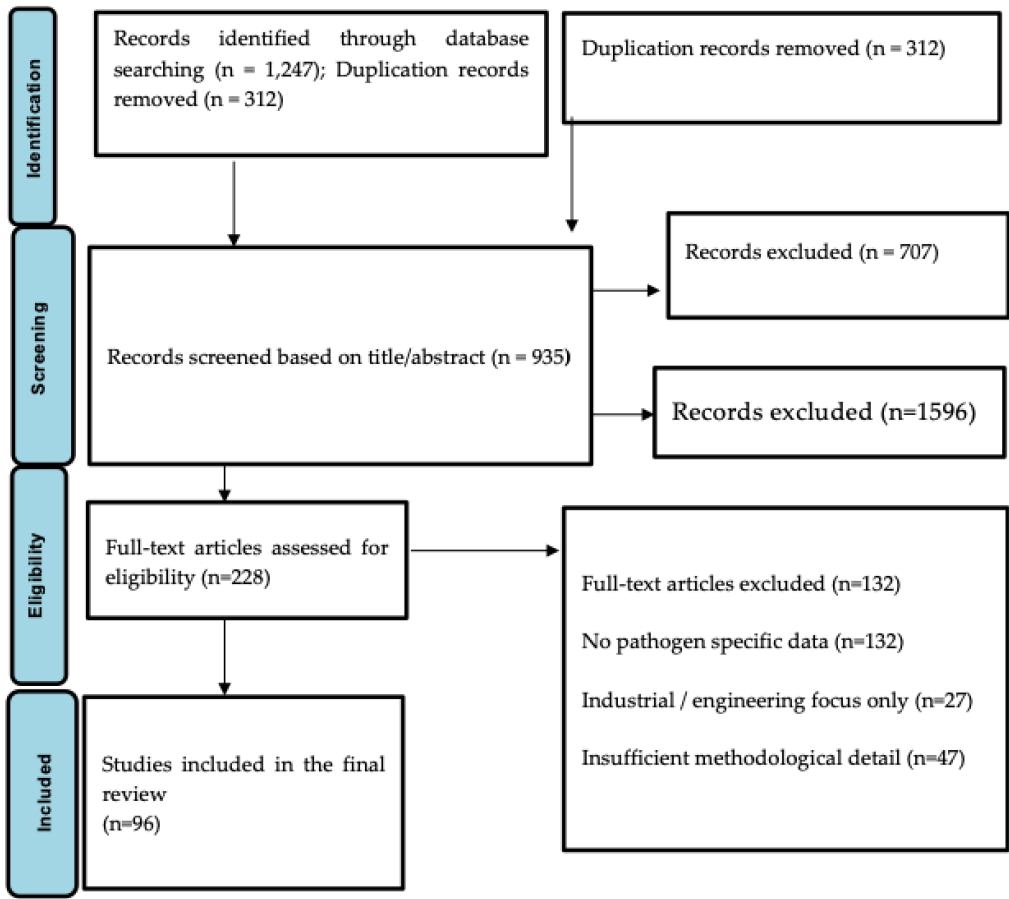


Figure 1. PRISMA Flow Chart: Details of Screening Procedure.

3.2. Detection of Gastrointestinal Pathogens in Wastewater

Multiple studies have revealed that wastewater is a reliable and informative matrix for the detection of gastrointestinal pathogens circulating within communities. Viral agents frequently include norovirus, a leading global cause of acute gastroenteritis, rotavirus, which is mainly prevalent among children under five years of age, and hepatitis A virus, especially during community-level outbreaks. In addition to viral pathogens, wastewater surveillance has constantly detected a range of enteric bacteria such as *Escherichia coli*, *Campylobacter spp.*, *Salmonella spp.*, and *Vibrio cholerae*. Protozoan pathogens, including *Cryptosporidium* and *Giardia*, have also been identified, further highlighting the capacity of wastewater-based epidemiology to apprehend diverse gastrointestinal pathogens of public health importance. Discovery rates vary by pathogen type, environmental conditions, and sampling approach, but wastewater monitoring consistently classifies pathogen circulation even when clinical reporting is low.

Table 1 provides a consolidated outline of wastewater-based epidemiology (WBE) initiatives conducted across the African continent. These studies vary extensively in scope from SARS-CoV-2 surveillance and poliovirus environmental monitoring to antimicrobial resistance (AMR) recognition and enteric virus tracking. The table highlights the variety of pathogens monitored, methodological platforms used, and the uneven distribution of WBE activities across countries. It also highlights the growing role of WBE as a complementary surveillance tool in resource-limited settings.

**Table 1.** Detection of Gastrointestinal Pathogens in Wastewater.

| Pathogen Group      | Specific Pathogens                                                      | Preferred Detection Method(s) | Reference                                                                   |
|---------------------|-------------------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------|
| Enteric viruses     | Norovirus, Rotavirus, Enteroviruses, HAV                                | RT-qPCR, ddPCR                | La Rosa <i>et al.</i> , 2019 [19]<br>; Hellmér <i>et al.</i> , 2014 [18]    |
| Bacterial pathogens | AMR pathogens in sewage, Salmonella spp., Shigella spp., Vibrio cholera | Culture, qPCR, sequencing     | Van Zyl <i>et al.</i> , 2017 [44]<br>Hendrick sen <i>et al.</i> , 2019 [81] |
| Protozoa            | Giardia, Cryptosporidium                                                | Immunofluorescence, qPCR      | Kitajima <i>et al.</i> , 2020 [14]                                          |
| AMR markers         | blaCTX-M, blaNDM, macrolide-resistance genes                            | qPCR, metagenomic sequencing  | Hendriksen <i>et al.</i> , 2019 [81]                                        |

Table 2 summarizes peer-reviewed studies that applied wastewater-based epidemiology methods across African countries, including study focus, pathogens detected, analytical platforms, and reference sources. It synthesizes key gastrointestinal pathogens detected in wastewater and the rational platforms commonly used for their identification. Enteric viruses such as norovirus, rotavirus, and adenovirus remain the most reported organisms due to their high shedding rates and environmental stability. Bacterial and protozoan pathogens need more specialized methods, including culture, PCR, and immunofluorescence assays. This table provides a technical foundation for understanding how wastewater reflects community-level circulation of GI diseases.

**Table 2.** Summarizes Major Pathogens Detectable in Wastewater.

| Country      | Study Focus                                        | Pathogens Detected                   | Analytical Platform       | Reference                                  |
|--------------|----------------------------------------------------|--------------------------------------|---------------------------|--------------------------------------------|
| Rwanda       | SARS-CoV-2 wastewater surveillance and feasibility | SARS-CoV-2 (RNA)                     | RT-qPCR, passive sampling | Edson <i>et al.</i> , 2025 [36];           |
| South Africa | National WBE network for COVID-19                  | SARS-CoV-2, PMMoV                    | RT-qPCR, normalization    | Street <i>et al.</i> , 2024 [41]           |
| Nigeria      | Enteric virus detection                            | Enteroviruses, adenovirus, norovirus | RT-PCR, cell culture      | Awolusi <i>et al.</i> , 2023 [58]          |
| Senegal      | Fecal sludge/ Viral persistence                    | Enteric and respiratory viruses      | RT-qPCR                   | Mendoza Grijalya <i>et al.</i> , 2024 [43] |
| Egypt        | Detection of HAV and HEV viruses                   | HAV, HEV                             | RT-PCR                    | Elmahdy <i>et al.</i> , 2019 [59]          |
| South Africa | Bacterial pathogen tracking                        | Salmonella, Shigella, Vibrio         | Culture + PCR             | Van Zyl <i>et al.</i> , 2017 [44]          |

3.3. Detection of Respiratory Pathogens in Wastewater

Respiratory virus detection in wastewater has increased rapidly since the COVID-19 pandemic, providing critical insights into community-level transmission dynamics. Several studies have showed that SARS-CoV-2 RNA is highly detectable in wastewater and demonstrates strong correlations with reported clinical case trends. Importantly, wastewater viral signals have normally been observed to precede clinical case surges by approximately 4 to 10 days, underscoring the early-

warning potential of wastewater-based epidemiology. In addition to SARS-CoV-2, influenza viruses (A and B) have also been effectively identified in untreated wastewater, further supporting the feasibility of wastewater surveillance for monitoring respiratory virus circulation beyond pandemic settings. These findings underscore the relevance of WBE beyond gastrointestinal pathogens, supporting integrated multi-pathogen surveillance systems.

3.4. Correlations Between Wastewater Signals and Clinical Case Data

Strong correlations between pathogen concentrations measured in wastewater and reported clinical cases have been known across diverse geographic regions. Wastewater signals of SARS-CoV-2 have been shown to closely track, and frequently forecast, community infection trends. Likewise, increases in norovirus RNA concentrations in wastewater coincide with gastroenteritis outbreaks, while rotavirus detection patterns align with seasonal trends in pediatric disease. Detection of hepatitis A virus in sewage has also been reported to precede clinical case spikes, enabling early outbreak identification. These correlations reinforce the value of wastewater-based epidemiology as a sensitive and timely population-level surveillance tool for infectious disease monitoring.

The respiratory pathogens identified in wastewater globally and in Africa, including methods used and representative studies.

The Table 3 summarizes respiratory pathogens that have been effectively identified in wastewater and stresses the rationale for their environmental detectability. SARS-CoV-2 remains the most documented respiratory virus in wastewater systems, but emerging evidence shows that influenza viruses, RSV, and adenoviruses can also be perceived. This demonstrates the expanding utility of WBE beyond enteric diseases, offering potential for integrated respiratory pathogen surveillance, especially during outbreaks or seasonal peaks. Also, a summary comparison of RT-qPCR and sequencing approaches is presented in Table 3.

Table 3. Detection of Respiratory Pathogens in Wastewater.

| Pathogen         | Rationale for Wastewater Detection           | Analytical Platform        | Reference                                           |
|------------------|----------------------------------------------|----------------------------|-----------------------------------------------------|
| SARS-CoV-2       | Fecal shedding; mucosal shedding             | RT-qPCR, ddPCR, sequencing | Kitajima et al., 2020 [14]; Edson et al., 2025 [36] |
| Influenza A/B    | Fecal shedding in infected individuals       | RT-qPCR                    | Wolfe et al., 2022 [85]                             |
| RSV              | Presence in stool and respiratory excretions | RT-qPCR                    | Wolfe et al., 2022 [53]                             |
| Human adenovirus | Dual GI and respiratory involvement          | qPCR                       | La Rosa et al., 2019 [19]                           |

3.5. Technical and Operational Considerations

The quality and reliability of wastewater-based epidemiology data are influenced by several interrelated methodological factors. These involve the choice of sampling strategy, such as grab versus composite sampling and the use of raw sewage linked with sludge, which can affect pathogen recovery and temporal representativeness. RNA preservation practices and viral decay dynamics throughout collection, transport, and storage further influence detection sensitivity. Also, variations in extraction and concentration workflows can considerably impact nucleic acid yield and analytical performance. The selection of molecular detection methods, including RT-qPCR, droplet digital PCR (ddPCR), and metagenomic sequencing, Finally, normalization approaches, such as the use of fecal indicators (e.g., PMMoV), wastewater flow data, or chemical tracers, are critical for improving comparability across sampling sites and over time.

Examples of studies demonstrating the strength of correlation between wastewater pathogen concentrations and reported clinical cases. This table presents examples of strong correlations between wastewater pathogen concentrations and reported clinical cases within different settings.

Several studies consistently show that wastewater signals can lead increases in clinical incidence by days or weeks, highlighting the value of WBE as an early warning system. This correlation strengthens the case for integrating WBE into national disease surveillance frameworks, particularly in settings where diagnostic access is limited (Table 4).

Table 4. Correlation Between Wastewater Signals and Clinical Cases.

| Pathogen    | Correlation Outcome                                                | Setting             | Reference                                                             |
|-------------|--------------------------------------------------------------------|---------------------|-----------------------------------------------------------------------|
| SARS-CoV-2  | Wastewater signal increases precede clinical cases by 4 to 14 days | Rwanda, Europe, USA | Huisman <i>et al.</i> , 2022 [31];<br>Edson <i>et al.</i> , 2025 [36] |
| Norovirus   | Wastewater peaks reflect seasonal occurrences                      | Sweden              | Hellmér <i>et al.</i> , 2014 [18]                                     |
| Polio       | Strong correlation between wastewater and AFP surveillance         | Global (WHO)        | WHO poliovirus guidelines, 2022 [49]                                  |
| Hepatitis A | Strong correlation between wastewater and AFP surveillance         | Japan, Italy        | La Rosa <i>et al.</i> , 2019 [19]                                     |

Comparison of major advantages and limitations of wastewater-based epidemiology in public-health surveillance. This table strengths and Limitations of WBE. Table 5 outlines the major strengths and limitations of WBE as reported in the scientific literature. While wastewater surveillance offers early detection, population-wide coverage, and the capability to capture asymptomatic infections, it also faces methodological and operational challenges. These include viral decay, infrastructure variability, and limitations in quantification methods. Understanding these advantages and constraints is essential for designing feasible and impactful WBE programs in low-resource contexts

Table 5. Strengths and Limitations of WBE.

| Category    | Key Points                                                                                  | Reference                        |
|-------------|---------------------------------------------------------------------------------------------|----------------------------------|
| Strengths   | Early detection, population-wide coverage, cost-effective, includes asymptomatic infections | Medema <i>et al.</i> , 2021 [37] |
| Limitations | Infrastructure gaps, variable viral decay, quantification challenges, limited lab capacity  | Wade <i>et al.</i> , 2022 [79]   |

This Table 6 highlights key opportunities for advancing wastewater-based epidemiology in low-resource African settings. These opportunities involve the adoption of passive sampling technologies, integration with Africa CDC genomic networks, improved urban sanitation systems, and enhanced laboratory and workforce capacity. Together, these dynamics create a pathway for sustainable WBE expansion and its incorporation into routine public health decision-making.

Table 6. Opportunities for WBE Implementation in Low-Resource African Settings.

| Opportunity Area                     | Description                                             | Reference                        |
|--------------------------------------|---------------------------------------------------------|----------------------------------|
| Passive sampling                     | Low-cost, suitable for irregular sewage systems         | Liu <i>et al.</i> , 2022 [89]    |
| Integration with Africa CDC networks | Strengthening surveillance through genomics initiatives | Africa CDC, 2023 [90]            |
| Urban sanitation expansions          | Improving coverage in cities such as Kigali, Nairobi    | UN Urbanization Report 2022 [49] |

|                                     |                                             |                                      |
|-------------------------------------|---------------------------------------------|--------------------------------------|
| Workforce and lab capacity-building | Training in PCR, sequencing, AMR monitoring | Hendriksen <i>et al.</i> , 2019 [81] |
|-------------------------------------|---------------------------------------------|--------------------------------------|

3.6. Regional WBE Evidence in Africa Compared with Findings from Rwanda

To position the findings of this study within the broader African context, Table 7 (Summary Table Wastewater-Based Epidemiology Studies in Africa) provides a consolidated overview of WBE initiatives conducted across the continent. These studies demonstrate how wastewater surveillance has been applied to detect a wide range of pathogens, including SARS-CoV-2, poliovirus, enteric viruses, antimicrobial-resistant bacteria, and viral hepatitis, using diverse analytical platforms such as RT-qPCR, cell culture, PEG precipitation, metagenomic sequencing, and passive environmental sampling. The regional indication highlights numerous trends relevant to considerate Rwanda’s progress. Countries with long-standing environmental surveillance systems such as South Africa, Nigeria, and Senegal have leveraged their existing laboratory networks to rapidly integrate SARS-CoV-2 wastewater monitoring, accomplishing strong correlations with clinical case trends and generating real-time public health insights. In contrast, countries such as Ghana, Kenya, Uganda, and Ethiopia have applied WBE to target specific public health priorities, including enteric viruses, antimicrobial resistance, and bacterial pollution in wastewater systems.

Alongside this background, Rwanda’s recent expansion of WBE activities aligns with wider regional improvements while also demonstrating notable innovation. Rwanda’s community wastewater surveillance, joined with the development of WBE frameworks for early outbreak detection and the combination of airport-based genomic surveillance using aircraft wastewater, places the country among Africa’s emerging leaders in leveraging wastewater as a strategic public health tool. These initiatives are particularly significant in a situation where conventional clinical surveillance may face constraints related to access, testing coverage, and resource limitations.

Largely, the evidence summarized in Table 7 shows that WBE is gradually recognized across Africa as a complementary and cost-effective surveillance approach. Comparing Rwanda’s experience with other countries stresses both combined challenges such as laboratory capability, infrastructure variability, and resource constraints and substantial opportunities for strengthening national and regional disease early-warning systems through consistent WBE practices, integrated genomic surveillance, and cross-country knowledge exchange.

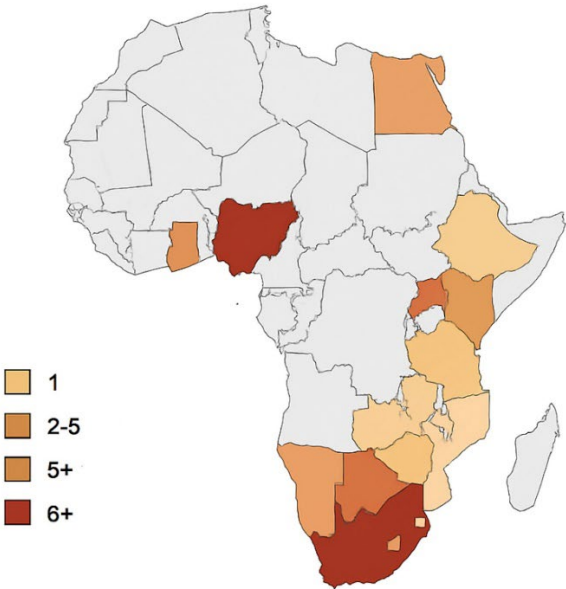
Table 7. Wastewater-Based Epidemiology (WBE) Studies in Africa.

| Country      | Research topic                                                        | Disease/Pathogen identified | Papers | Platform used                                    | Scientific References                                                                                    |
|--------------|-----------------------------------------------------------------------|-----------------------------|--------|--------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| South Africa | SARS-CoV-2 detection in municipal wastewater                          | COVID-19                    | 6+     | RT-qPCR, Sequencing (illumine) PEG concentration | Johnson R. <i>et al.</i> , 2021 [50]; Lester <i>et al.</i> , 2022 [51]; Street <i>et al.</i> , 2024 [52] |
|              | Poliovirus environmental surveillance                                 | Polio (WPV, cVDPV)          | 3      | Cell culture, RT-PCR                             | Mahlangu <i>et al.</i> , 2019 [53]; Gumede <i>et al.</i> , 2020 [54]                                     |
|              | AMR genes in wastewater                                               | Antimicrobial resistance    | 2      | Metagenomics, qPCR                               | Nkado R. <i>et al.</i> , 2020 [55]                                                                       |
| Nigeria      | Poliovirus environmental surveillance (national surveillance program) | Polio                       | 5+     | PCR, cell culture                                | Adeniji <i>et al.</i> , 2017 [56]; Baba <i>et al.</i> , 2018 [57];                                       |

|          |                                                                               |                                                     |                                                                  |                                                                         |                                                                                                  |
|----------|-------------------------------------------------------------------------------|-----------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Egypt    | SARS-CoV-2 in wastewater                                                      | COVID-19                                            | 1                                                                | RT-qPCR                                                                 | Awolusi et al., 2023 [58]                                                                        |
|          | Hepatitis A&E monitoring in wastewater                                        | HAV, HEV                                            | 2                                                                | RT-PCR                                                                  | El-Mahdy et al., 2019 [59]; El-Said et al., 2020 [60]                                            |
|          | SARS-CoV-2 detection                                                          | COVID-19                                            | 1                                                                | RT-qPCR                                                                 | Shawky et al., 2021 [61]                                                                         |
| Tunisia  | SARS-CoV-2 detection in wastewater                                            | COVID-19                                            | 2                                                                | RT-qPCR;                                                                | Barhoumi et al., 2021 [62]; Wurtzer et al., 2021 [63]                                            |
| Morocco  | Surveillance of SARS-CoV-2 in urban wastewater                                | COVID-19                                            | 1                                                                | RT-qPCR                                                                 | Nasseri et al., 2021 [64]                                                                        |
| Kenya    | Antibiotic resistance bacteria in wastewater                                  | AMR bacteria (E. Coli, Klebsiella, Salmonella       | 2                                                                | Culture, AST, PCR                                                       | Kiiru et al., 2018 [65]; Omwandho et al., 2019 [66];                                             |
|          | SARS-CoV-2 wastewater surveillance                                            | COVID-19                                            | 1                                                                | RT-qPCR                                                                 | Oyaro et al., 2021 [67]                                                                          |
| Uganda   | AMR monitoring in wastewater                                                  | Resistance E. Coli & Enterococcus                   | 1                                                                | Culture based, PCR                                                      | Katongole et al., 2020 [68]                                                                      |
| Ghana    | Environmental detection of enteric viruses                                    | Norovirus, Rotavirus, Adenovirus                    | 2                                                                | RT-PCR                                                                  | Armah et al., 2016 [69]; Aboagye et al., 2018 [70]                                               |
| Senegal  | Polio environmental surveillance                                              | Polioviruses                                        | 2                                                                | Cell culture, PCR                                                       | Diop et al., 2014 [71]; Mendoza et al., 2024 [72]                                                |
| Zimbabwe | SARS-CoV-2 surveillance                                                       | COVID-19                                            | 1                                                                | RT-qPCR                                                                 | Manangazira et al., 2021 [73]                                                                    |
| Rwanda   | SARS-CoV-2 detection in municipal wastewater                                  | COVID-19                                            | 2                                                                | RT-qPCR, viral concentration (PEG) Sequencing support via regional labs | Uwimana A. et al., 2022 [74]; University of Rwanda - RBC wastewater monitoring report, 2021 [75] |
|          | Development of wastewater surveillance framework for early outbreak detection | Multi-pathogen (SARS-CoV-2) enteric viruses         | 1                                                                | RT-qPCR, environmental sampling protocol                                | Ndayishimiye et al., 2023 [76]                                                                   |
|          | Airport-based WBE and genomic surveillance using aircraft wastewater and      | SARS-CoV-2 and variants and other imported lineages | ≥2 peer-reviewed genomic surveillance studies using wastewater & | Aircraft wastewater sampling + pooled nasal swabs; RT-qPCR and whole-   | Edson R et al., 2025 [36]; (Misbah G et al., 2025 [77]                                           |

|          | pooled nasal<br>swabs from<br>international<br>travelers at<br>Kigali<br>International<br>Airport |                                 | swabs at the<br>airport | genome<br>sequencing to<br>detect/imported<br>variants;<br>integration with<br>national<br>genomic<br>surveillance |                                        |
|----------|---------------------------------------------------------------------------------------------------|---------------------------------|-------------------------|--------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| Ethiopia | AMR genes                                                                                         | ARGs (blaCTX-<br>M, mecA, etc.) | 1                       | qPCR                                                                                                               | Alemayehu <i>et al.</i> ,<br>2020 [78] |

Figure 2, in combination with Table 7, show the geographic distribution, thematic focus, and methodological diversity of wastewater-based epidemiology (WBE) studies conducted across Africa. The figure highlights that WBE activities have been concentrated in a limited number of countries, with South Africa, Nigeria, and Rwanda emerging as regional leaders in the implementation of wastewater surveillance. South Africa demonstrates the most expanded WBE portfolio, including SARS-CoV-2 surveillance, long-standing poliovirus environmental surveillance, and antimicrobial resistance (AMR) monitoring using advanced molecular platforms, with RT-qPCR, metagenomic sequencing, and PEG-based concentration methods [50–55]. Several countries, including Nigeria, Senegal, and Ghana, have leveraged WBE mainly for enteric virus surveillance, particularly poliovirus and other gastrointestinal pathogens, reflecting alignment with global polio eradication and diarrheal disease control initiatives [56,57,69–72]. In contrast, North African countries such as Egypt, Tunisia, and Morocco have focused predominantly on SARS-CoV-2 and hepatitis virus detection in wastewater, largely adopting RT-qPCR-based approaches through the COVID-19 pandemic [59–64]. East African countries, including Kenya, Uganda, Ethiopia, and Rwanda, demonstrate an increasing integration of WBE into antimicrobial resistance surveillance and pandemic awareness. Kenya and Uganda have applied culture-based approaches combined with PCR to reveal resistant *Escherichia coli*, *Enterococcus*, and other clinically significant bacteria [65–68], while Ethiopia has focused on quantifying resistance genes using qPCR [78]. Rwanda presents a particularly notable case, with several complementary WBE initiatives ranging from municipal SARS-CoV-2 surveillance to the development of national wastewater surveillance frameworks and innovative airport-based wastewater and genomic surveillance targeting imported variants at Kigali International Airport [74–77].



**Figure 2.** Wastewater-based epidemiology studies in Africa.

## 4. Discussion

### 4.1. Overview of Wastewater-Based Epidemiology (WBE) for GI and Respiratory Pathogens

Wastewater-based epidemiology (WBE) has appeared as a powerful population-level surveillance tool capable of detecting a broad spectrum of gastrointestinal (GI) and respiratory pathogens shed in human excretion. Pathogens excreted in feces, urine, saliva, sputum, and mucus enter sewage networks, allowing the aggregation of biological signals from large communities regardless of healthcare-seeking behavior or testing access [14,35,38]. WBE complements traditional surveillance by providing near-real-time data on pathogen flows, including viruses (e.g., norovirus, rotavirus, adenovirus, SARS-CoV-2), bacteria (e.g., *Salmonella*, *Shigella*, *Vibrio*), and antimicrobial resistance (AMR) markers [44,80,81]. Its application expanded substantially during the COVID-19 pandemic and is currently recognized as a scalable early-warning approach, particularly valuable for resource-limited settings where routine clinical surveillance remains fragmented [36,37,79].

### 4.2. Detection of Gastrointestinal Pathogens in Wastewater

Various studies have demonstrated successful detection of GI pathogens in wastewater using RT-qPCR, ddPCR, culture-based assays, and sequencing. Enteric viruses such as norovirus, rotavirus, hepatitis A virus (HAV), and enteroviruses are within the most frequently monitored and consistently detected at high concentrations due to robust fecal shedding dynamics [18,19,36,48,50]. Bacterial pathogens including *Salmonella enterica*, *Vibrio cholerae*, *Shigella* spp., *Campylobacter* spp., and diarrheagenic *Escherichia coli* have been identified across various wastewater systems globally, including in African settings such as South Africa, Nigeria, Senegal, and Rwanda [43,44,83,84]. Wastewater has also proven valuable for identifying AMR genes (e.g., blaCTX-M, carbapenemases, macrolide resistance markers), offering insights into community-level resistance pressures [49]. These findings highlight WBE's strong potential to track GI diseases that represent a major burden across Africa.

### 4.3. Detection of Respiratory Pathogens in Wastewater

Although respiratory pathogens are not naturally associated with fecal-oral transmission, several respiratory viruses, including SARS-CoV-2, influenza A/B, respiratory syncytial virus (RSV), and human metapneumovirus have been consistently detected in wastewater due to mucosal shedding and enteric replication pathways [14,37,86]. SARS-CoV-2 surveillance via WBE has been broadly validated, including in low-resource settings such as Kigali, where viral RNA trends strongly revealed clinical case patterns during multiple pandemic waves [36,86]. Emerging studies also exhibit wastewater detection of influenza viruses and RSV, enabling population-level monitoring even when clinical testing readiness decreases or asymptomatic infections dominates transmission [85,87]. These advances swell the scope of WBE from traditional GI pathogens toward broader respiratory disease surveillance.

### 4.4. Correlation Between Wastewater Signals and Clinical Data

A reliable finding across global literature is the strong correlation between wastewater pathogen concentrations and reported clinical case data. For SARS-CoV-2, multiple studies demonstrated that growths in wastewater RNA concentrations precede rises in clinical cases by 4 to 14 days, making WBE a dependable early-warning indicator [31,36,37,86]. Similar correlations have been observed for norovirus, rotavirus, poliovirus, hepatitis A, and *Salmonella* outbreaks, where wastewater detection also predicted or reflected community transmission dynamics [18,82]. In Rwanda, wastewater SARS-CoV-2 concentrations measured at major treatment facilities in Kigali showed high concordance with PCR-confirmed case data throughout the pandemic [86]. This alignment emphasizes WBE's utility in settings with limited diagnostic coverage, asymptomatic infections, or delays in clinical reporting.

#### 4.5. Strengths and Limitations of WBE in Public Health Surveillance

Wastewater-based epidemiology (WBE) offers several strengths that make it a valuable tool for communicable disease surveillance. It is a cost-effective surveillance approach, particularly in low-resource settings where widespread individual testing is not feasible [44,87]. In addition, WBE allows the simultaneous monitoring of multiple pathogens, including antimicrobial resistance markers, and offers a noninvasive, community-wide surveillance mechanism that supports equity in public-health responses [81]. It also provides population-level coverage that is independent of healthcare-seeking behavior, acknowledging more comprehensive monitoring of community infection dynamics [35,38]. WBE can detect pathogen circulation early, often before symptomatic cases peak, thereby supporting suitable public-health interventions [31,37]. Despite these benefits, WBE also presents several limitations. Variability in sewage infrastructure can affect the representativeness of samples, particularly in informal or underserved settlements [35,44]. Environmental factors such as temperature, pH, flow rate, and hydraulic retention time influence the decay of RNA and DNA in wastewater, potentially decreasing detection sensitivity [37,80]. Further challenges include difficulties in accurate quantification, normalization, and establishing direct links between wastewater signals and precise case numbers [88,89]. Furthermore, limited laboratory capacity for molecular analysis and sequencing in many African regions constrains large-scale implementation [44,83]. However, when integrated with environmental, clinical, and genomic data, WBE remains a powerful and complementary approach to traditional surveillance systems, particularly for strengthening early warning and public health awareness.

#### 4.6. Implications for Early Warning Systems

WBE's capacity to identify pathogens before clinical increases occur makes it a cornerstone for early-warning systems. Through detecting pre-symptomatic and asymptomatic shedding, WBE can guide rapid interventions such as targeted assessment, vaccination campaigns, public-risk communication, and resource allocation [18,31,37]. During COVID-19, WBE allowed early detection of emerging variants and informed public-health decision-making even when clinical-testing capacity was overwhelmed [14,86]. Similar frameworks are now being applied to other pathogens such as cholera, norovirus, and AMR genes to support outbreak prediction and response strategies in Africa and other resource-limited regions [43,49,56]. Its integration into national surveillance systems can significantly increase resilience against future rises.

#### 4.7. Opportunities for Implementation in Low-Resource African Settings

African settings present a unique opening for scaling WBE due to the disproportionate burden of infectious diseases, under-resourced clinical surveillance systems, and increasing wastewater infrastructure in urban centers such as Kigali, Nairobi, Johannesburg, Dakar, and Lagos [43,44,83]. Passive samplers (e.g., Moore swabs) have demonstrated particularly effective and low-cost for wastewater monitoring in Rwanda and other low-resource environments [86,89]. Regional initiatives such as the Africa CDC's Pathogen Genomics Initiative and national early-warning agendas provide platforms for integrating WBE into routine disease surveillance and outbreak response frameworks [14,84,90]. Strengthening laboratory capacity, normalizing methods, and establishing cross-country data-sharing networks will be crucial to expanding WBE's role in pathogen surveillance across Africa. With suitable investment, WBE can become a sustainable and equitable surveillance tool supporting epidemic preparedness in diverse urban and peri-urban African communities.

### 5. Conclusions

Wastewater-based epidemiology provides a dominant, non-invasive approach for monitoring community-level circulation of gastrointestinal and respiratory pathogens. Evidence displays that wastewater signals often precede clinical cases, suggesting valuable early warning for outbreaks. WBE is especially valuable in resource-limited surroundings where diagnostic access is constrained.

Integrating WBE into national surveillance frameworks can enhance infectious disease monitoring, inform targeted interventions, and strengthen overall public health resilience. Continued investment in laboratory infrastructure, capacity building for early career researchers, standardized methodologies, and cross-sector collaboration will be essential to scaling WBE programs across Africa and globally.

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Abbreviations

The following abbreviations are used in this manuscript:

| Abbreviation | Definition                                          |
|--------------|-----------------------------------------------------|
| AMR          | Antimicrobial Resistance                            |
| AMS          | Antimicrobial Stewardship                           |
| API20E       | Analytical Profile Index 20 Enterobacterales        |
| ARGs         | Antimicrobial Resistance Genes                      |
| AST          | Antimicrobial Susceptibility Testing                |
| CLSI         | Clinical and Laboratory Standards Institute         |
| CSF          | Cerebrospinal Fluid                                 |
| CCN          | Clinical Case Notification                          |
| COVID-19     | Coronavirus Disease 2019                            |
| ddPCR        | Droplet Digital Polymerase Chain Reaction           |
| DNA          | Deoxyribonucleic Acid                               |
| DOAJ         | Directory of open access journals                   |
| ESBL         | Extended-Spectrum Beta-Lactamase                    |
| GI           | Gastrointestinal                                    |
| GLASS        | Global Antimicrobial Resistance Surveillance System |
| HAI          | Hospital-Acquired Infection                         |
| HAV          | Hepatitis A Virus                                   |
| HEV          | Hepatitis E Virus                                   |
| ICU          | Intensive Care Unit                                 |
| IPC          | Infection Prevention and Control                    |
| IRB          | Institutional Review Board                          |

|            |                                                                    |
|------------|--------------------------------------------------------------------|
| LMICs      | Low- and Middle-Income Countries                                   |
| MDRO       | Multidrug-Resistant Organism                                       |
| MDPI       | Multidisciplinary Digital Publishing Institute                     |
| Mesh       | Medical Subject Headings                                           |
| MIC        | Minimum Inhibitory Concentration                                   |
| MDRO       | Multidrug-Resistant Organism                                       |
| MS2        | MS2 Bacteriophage                                                  |
| PMMoV      | Pepper Mild Mottle Virus                                           |
| PRISMA     | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| qPCR       | Quantitative Polymerase Chain Reaction                             |
| RNA        | Ribonucleic Acid                                                   |
| RSV        | Respiratory Syncytial Virus                                        |
| RT-PCR     | Reverse Transcription Polymerase Chain Reaction                    |
| RT-qPCR    | Reverse Transcription Quantitative PCR                             |
| SARS-CoV-2 | Severe Acute Respiratory Syndrome Coronavirus 2                    |
| SSIs       | Surgical Site Infections                                           |
| UN DESA    | United Nations Department of Economic and Social Affairs           |
| WHO        | World Health Organization                                          |
| WBE        | Wastewater-Based Epidemiology                                      |
| WPV        | Wild Poliovirus                                                    |

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