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Research Paper

Understanding The Application of Multi-Pathogen Wastewater Surveillance in Health Emergency Preparedness and Response: A Systematic Review

Akpa Igwe Chijioke
Nigeria Centre for Disease Control and Prevention (NCDC)
ORCiD Number 0000 0003 0904 116x
Corresponding Author: Akpa Igwe Chijioke

Chinyere Happiness Nwani
African Institute for Health Policy and Health Systems

Okorie Ogbonnaya Matthew
African Institute for Health Policy and Health Systems

Rejoice Kudirat Luka-Lawal
Nigeria Centre for Disease Control and Prevention (NCDC)
ORCID: 0009-0004-9227-3141

Abiodun Ogunniyi
Nigeria Centre for Disease Control and Prevention (NCDC)
ORCID: 0000-0002-6625-9192

ABSTRACT
Multi-pathogen wastewater surveillance (MPWS) has emerged as a valuable population-level public health tool, particularly seen during the COVID-19 pandemic. While traditional wastewater surveillance often focuses on single pathogens, integrated approaches can enhance early detection and support health emergency preparedness. This study systematically reviewed global evidence on MPWS to examine its characteristics, pathogen scope, methodological approaches, and contributions to health emergency preparedness and response. A systematic search identified 532 records from peer-reviewed and grey literature. After removing duplicates and applying inclusion criteria, 32 studies were included. Data were synthesized narratively, focusing on geographic distribution, surveillance settings, study designs, pathogens monitored, analytical methods, linkages to emergency preparedness, and implementation challenges and enablers. The studies were globally distributed but concentrated in Europe (31.3%) and North America (25%), with lower representation from Africa and Latin America. Most studies (71.9%) monitored both viral and bacterial pathogens, while some also included antimicrobial resistance genes and protozoa. SARS-CoV-2 was the most frequently monitored pathogen, followed by poliovirus and enteric bacteria. Molecular methods, including RT-qPCR and metagenomic sequencing, dominated analytical approaches. MPWS consistently provided early detection of pathogens compared with clinical surveillance, supporting outbreak early warning, resource allocation, variant tracking, and public health communication. Implementation challenges included limited laboratory capacity, lack of standardized protocols, and weak integration into national surveillance systems, whereas cross-sectoral coordination, technological innovation, and policy support emerged as key enablers. Multi-pathogen wastewater surveillance is a versatile and effective tool for strengthening health emergency preparedness. Its integration into routine public health systems, supported by standardized protocols, workforce capacity building, and institutional commitment, can enhance early detection, inform timely interventions, and improve the resilience of health systems against emerging infectious disease threats.

Keywords: *Multi-pathogen, Wastewater, Surveillance, Emergency preparedness.*

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I. INTRODUCTION

Background of Study

Over the past two decades, the global community has experienced recurrent infectious disease outbreaks, including severe acute respiratory syndrome (SARS), Influenza A (H1N1), Ebola Virus Disease, and most recently Coronavirus Disease 2019 (COVID-19). These public health emergencies have revealed persistent weaknesses in conventional disease surveillance systems, which are largely dependent on clinical reporting and laboratory confirmation of cases (World Health Organization [WHO], 2020). Such systems are inherently reactive and often detect outbreaks only after sustained community transmission has occurred. In many low- and middle-income countries (LMICs), including Nigeria, these limitations are further compounded by inadequate diagnostic infrastructure, delayed health-seeking behavior, underreporting, and limited laboratory capacity (Adejumo *et al*., 2021; Oyeyemi *et al*., 2020). The COVID-19 pandemic demonstrated that reliance solely on clinical surveillance can result in delayed detection, thereby undermining timely public health response and control measures (Ahmed *et al*., 2020). Wastewater-based surveillance (WBS), also known as wastewater-based epidemiology (WBE), has emerged as a complementary and innovative approach to infectious disease monitoring. It involves the analysis of municipal wastewater to detect microbial biomarkers primarily DNA or RNA shed by infected individuals into sewer systems (Daughton, 2020). Because both symptomatic and asymptomatic individuals excrete pathogens, wastewater surveillance provides population-level data that accurately reflect community infection dynamics than clinical testing alone (Medema *et al*., 2020).

Historically, wastewater surveillance has been used successfully for environmental monitoring of poliovirus, contributing to polio eradication efforts in several countries, including Nigeria (Asghar *et al*., 2014; WHO, 2015). During the COVID-19 pandemic, wastewater surveillance gained unprecedented global relevance as numerous studies demonstrated its ability to detect SARS-CoV-2 RNA in sewage days or weeks before increases in clinically reported cases (Peccia *et al*., 2020; Wu *et al*., 2020). The application of advanced molecular tools such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS) has further enhanced the sensitivity and specificity of wastewater analysis (Crits-Christoph *et al*., 2021).

More recently, the scope of wastewater surveillance has expanded beyond single-pathogen detection to multi-pathogen wastewater surveillance, which simultaneously monitors viruses, bacteria, protozoa, and antimicrobial resistance (AMR) genes (Hendriksen *et al*., 2019; Polo *et al*., 2020). This approach aligns with global calls for integrated surveillance systems capable of supporting health emergency preparedness and response (WHO, 2022). In Nigeria and similar settings, multi-pathogen wastewater surveillance offers a cost-effective and scalable strategy for strengthening early warning systems, particularly in urban and peri-urban areas with limited access to routine diagnostic testing (Afolayan *et al*., 2023). Despite these advantages, the application of multi-pathogen wastewater surveillance remains uneven and poorly integrated into national preparedness frameworks. A systematic synthesis of existing evidence is therefore necessary to clarify its effectiveness, limitations, and policy relevance for health emergency preparedness and response.

In Nigeria and many other LMICs, infectious disease surveillance remains heavily reliant on facility-based reporting and laboratory-confirmed diagnoses. While these approaches are fundamental to public health practice, they often fail to detect outbreaks at an early stage, particularly when infections are asymptomatic or mildly symptomatic (NCDC, 2021). Delays in testing, limited laboratory coverage, and socioeconomic barriers to healthcare access further weaken the effectiveness of traditional surveillance systems. The COVID-19 pandemic highlighted these shortcomings, as widespread community transmission occurred before clinical surveillance systems were able to respond effectively (WHO, 2020). Although wastewater surveillance demonstrated significant value as an early warning tool during the pandemic, most efforts focused narrowly on SARS-CoV-2, with limited exploration of its broader potential for multi-pathogen detection and comprehensive health emergency preparedness (Ahmed *et al*., 2020).

Moreover, the implementation of wastewater surveillance faces several challenges, including lack of standardized methodologies, environmental variability affecting data interpretation, ethical and privacy concerns, and weak policy and regulatory frameworks. In Nigeria, these challenges are compounded by infrastructural deficits in sanitation systems and limited institutional integration of environmental surveillance into public health decision-making (Oyeyemi *et al*., 2020). The absence of a systematic review synthesizing evidence on the application of multi-pathogen wastewater surveillance for health emergency preparedness limits its uptake and effective utilization. Without such evidence-based understanding, public health authorities may continue to miss critical opportunities for early detection and proactive response to emerging infectious disease threats.

The aim of this study is to systematically review the application of multi-pathogen wastewater surveillance in health emergency preparedness and response, with emphasis on its role as an early warning system for infectious disease outbreaks.

II. METHODOLOGY

Research Design

This study of systematic review was designed to enable the systematic identification, evaluation, and synthesis of empirical and grey literature on multi-pathogen wastewater surveillance within the context of health emergency preparedness and response.

Review Questions

This review was guided by the following research questions:

i. What are the current applications of multi-pathogen wastewater surveillance in health emergency preparedness and response globally?
ii. Which pathogens are commonly monitored through wastewater surveillance in emergency contexts?
iii. What mechanisms, laboratory methodologies, and technologies are used in multi-pathogen detection within wastewater systems?
iv. What benefits, challenges, and limitations are associated with integrating wastewater surveillance into health emergency preparedness frameworks?

Search Strategy

A comprehensive search strategy was developed to identify relevant literature published between 2010 and 2024. This period reflects contemporary advancements in wastewater-based epidemiology (WBE), including the expanded application of pathogen surveillance during public health crises such as Ebola outbreaks, antimicrobial resistance surveillance, and the COVID-19 pandemic. Searches were conducted using the following electronic databases: Google Scholar; PubMed; Scopus; Web of Science; JSTOR; African Journals Online (AJOL)

The search strategy combined keywords, subject headings, and Boolean operators. Primary search terms included: wastewater surveillance, multi-pathogen detection, wastewater-based epidemiology, environmental surveillance, public health emergencies, disease outbreaks, pathogen monitoring, preparedness, response. Secondary modifiers included: enteric viruses, SARS-CoV-2, poliovirus, antimicrobial resistance, cholera, typhoid fever, Ebola, norovirus, influenza, and microbial indicators. Grey literature was also reviewed to capture non–peer-reviewed programmatic evidence.

Inclusion and Exclusion Criteria

Inclusion Criteria:

i. Studies, reports, and program documents addressing wastewater surveillance for two or more pathogens.
ii. Literature focused on health emergency preparedness, outbreak monitoring, or response frameworks.
iii. Publications between 2010 and 2024.
iv. Studies written in English.
v. Empirical studies, technical assessments, program evaluations, and surveillance guidelines.

Exclusion Criteria:

i. Studies focused exclusively on single-pathogen wastewater surveillance without relevance to emergency preparedness.
ii. Publications unrelated to disease surveillance, laboratory detection, or public health response.
iii. Opinion pieces or commentaries lacking empirical or documented methodological evidence.
iv. Studies not linked to environmental surveillance or wastewater sampling approaches.

Study Selection Process

The study selection process followed a structured and transparent workflow. Retrieved records from all databases were imported into a reference management software, and duplicate records were removed. Title and abstract screening were conducted against the predefined inclusion and exclusion criteria. Full texts of potentially relevant articles were retrieved and assessed for eligibility. Where uncertainty arose, articles were re-examined, and consensus was reached through iterative review of the criteria.

The selection process involved four phases:

i. **Identification**: Retrieval of studies from databases and grey literature.
ii. **Screening**: Title and abstract review to determine relevance.

iii. **Eligibility**: Full-text evaluation based on methodological and thematic alignment.
iv. **Inclusion**: Final determination of studies for narrative synthesis.

Data Extraction

A structured extraction matrix was developed to ensure uniformity in data collection across studies. The following information was extracted: Author(s) and publication year; Country/region of surveillance implementation; Target pathogens and number of pathogens monitored; Wastewater sampling and laboratory detection methodologies; Surveillance design and operational; Linkages to emergency preparedness or response systems; Strengths, gaps, and implementation outcomes; Recommendations and lessons learned

Data Synthesis

A thematic narrative synthesis approach was employed to integrate findings across studies. Consistent with the framework of Popay *et al*. (2006), synthesis proceeded through the following stages:

i. **Preliminary synthesis**: Organizing extracted data to identify patterns in surveillance methodologies, pathogen coverage, and emergency use cases.
ii. **Exploring relationships:** Examining interrelationships between pathogen detection capacity, surveillance environments, technological sophistication, resource availability, and emergency response integration.
iii. **Assessing synthesis robustness:** Cross-referencing evidence strength, methodological quality, and consistency across included literature.

Core themes analyzed included technological capacity, detection sensitivity, operational infrastructure, inter-agency coordination, outbreak early warning potential, data integration challenges, and policy uptake.

Ethical Considerations

The review relied exclusively on published and publicly accessible literatures; hence, ethical approval was not required. Nevertheless, ethical standards were maintained by acknowledging all information sources, safeguarding author intellectual property through proper citation, and ensuring that findings were presented accurately and without misrepresentation.

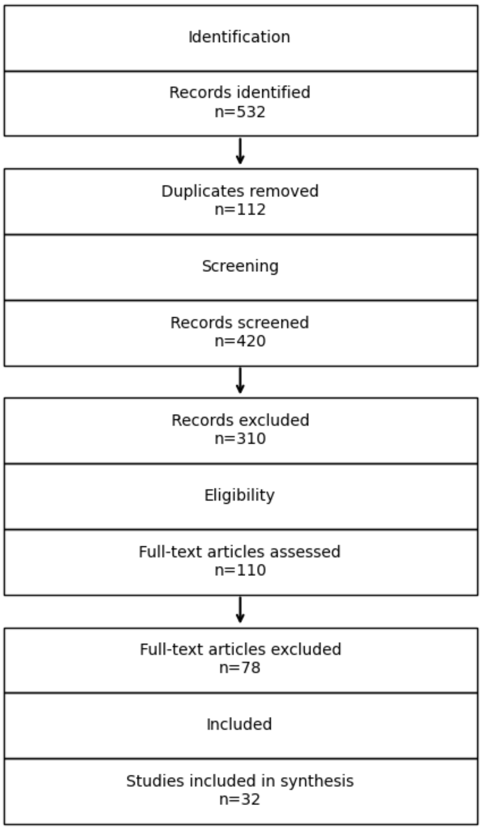

Figure 1: PRISMA Diagram showing the Identification, Screening, Eligibility, and Inclusion of Studies

III. RESULTS

The result of this systematic review revealed that 532 records was identified from databases and grey literature. After 112 duplicates were removed, 420 records were screened by title and abstract, leading to the exclusion of 310 studies that were not relevant to multi-pathogen wastewater surveillance or health emergency preparedness. Full-text assessment was conducted for 110 articles, of which 78 were excluded due to limited methodological detail, a narrow single-pathogen focus, or weak links to emergency preparedness. In total, 32 studies met the inclusion criteria and were included in the narrative synthesis.

Geographic Distribution, Surveillance Settings, and Study Designs of Included Studies

Table 1A shows that the included studies were globally distributed but unevenly represented across regions. Europe contributed the largest share (31.3%), followed by North America (25.0%) and Asia (18.8%). Africa (12.5%), Oceania (6.2%), and Latin America (6.2%) were less represented, highlighting gaps in evidence from regions that often face higher infectious disease risks.

The studies were conducted in varied surveillance settings, including municipal wastewater treatment plants, informal settlements, and mobile sampling units used during outbreaks. This reflects the flexibility of multi-pathogen wastewater surveillance across both centralized systems and emergency or low resource (Table 1B). In terms of study design, all major approaches were represented, including cross-sectional assessments, long-term monitoring, outbreak investigations, laboratory evaluations, and implementation-focused analyses (Table 1C). This indicates a broad and evolving evidence base that addresses both technical performance and real-world application of wastewater surveillance systems.

Table 1: Geographic Distribution, Surveillance Settings, and Study Designs of Included Studies (n = 32)

Table 1A. Geographic Distribution of Studies

Region	Frequency (n)	Percentage (%)
Europe	10	31.3
North America	8	25.0
Asia	6	18.8
Africa	4	12.5
Oceania	2	6.2
Latin America	2	6.2
Total	**32**	**100.0**

Table 1B. Surveillance Settings Reported (Multiple Responses)

Surveillance Setting	Reported
Municipal wastewater treatment plants	Yes
Informal settlements	Yes
Mobile sampling units during outbreaks	Yes

Table 1C. Study Designs Included (Multiple Responses)

Study Design	Reported
Cross-sectional assessments	Yes
Long-term monitoring programs	Yes
Outbreak investigations	Yes
Laboratory method evaluations	Yes
Implementation-focused analyses	Yes

Characteristics of Included Studies

Table 2 shows that most of the included studies had a broad surveillance scope, with nearly three-quarters (71.9%, n = 23) monitoring both viral and bacterial pathogens. This reflects a strong emphasis on integrated, multi-pathogen approaches rather than single-disease surveillance. The remaining studies (28.1%, n = 9) extended surveillance to other indicators such as antimicrobial resistance genes, protozoa, and fungal markers, highlighting efforts to capture a wider range of public health threats.

In terms of duration, the typical surveillance period was about two years, suggesting that many studies were designed to generate medium-term evidence for public health planning. However, the presence of long-running programs lasting more than ten years indicates that wastewater surveillance can be sustained over time and used for continuous population-level monitoring. Overall, the table illustrates both the growing adoption of comprehensive pathogen monitoring and the potential for long-term wastewater surveillance as a reliable public health tool.

Table 2: Characteristics of Included Studies (n = 32)

Aspect	Category	Frequency (n)	Percentage (%)	Description
Pathogens Monitored	Viral and bacterial pathogens	23	71.9	Studies monitoring both viral and bacterial targets
	Other indicators	9	28.1	AMR genes, protozoa, and fungal indicators
Study Duration	Typical duration	—	—	Approximately 2 years
	Long-term surveillance	—	—	Continuous monitoring exceeding 10 years

Pathogens Monitored in Multi-Pathogen Surveillance

Multi-pathogen wastewater surveillance captured a breadth of microbial indicators relevant to health emergencies. Viral pathogens were the most frequently monitored (SARS-CoV-2, Poliovirus, Norovirus, Rotavirus, Adenovirus, Influenza), followed by gastrointestinal bacteria (Vibrio cholerae, Salmonella Typhi, Shigella spp.), antimicrobial resistance determinants (NDM-1, ESBL genes), and protozoa (Giardia, Cryptosporidium).

SARS-CoV-2 appeared in 81 percent of the included studies, reflecting global prioritization during the COVID-19 pandemic, while poliovirus monitoring remained central in low-resource and polio-endemic contexts to support eradication initiatives. A subset of studies combined pathogen surveillance with quantification of AMR genes, demonstrating integrated biosurveillance capability.

Table 3: Pathogens Monitored in Multi-Pathogen Wastewater Surveillance Studies

Pathogen Group	Specific Pathogens Monitored	Frequency of Monitoring Across Included Studies	Key Observations and Relevance to Health Emergencies
Respiratory & Enteric Viruses	SARS-CoV-2, Poliovirus, Norovirus, Rotavirus, Adenovirus, Influenza viruses	SARS-CoV-2 reported in ~81% of studies; other viruses vary by geographic setting and research focus	Viral surveillance dominated due to COVID-19; SARS-CoV-2 provided real-time outbreak intelligence; Poliovirus consistently monitored in polio-endemic regions to support eradication campaigns
Enteric Bacteria	Vibrio cholerae, Salmonella Typhi, Shigella spp.	Moderate frequency across LMIC-based studies; particularly where waterborne disease burden is high	Detection informs outbreak preparedness for cholera and typhoid; supports early detection during seasonal peaks and post-flood events
Antimicrobial Resistance (AMR) Determinants	NDM-1, ESBL-encoding genes, carbapenemase genes	Included in a subset of multi-pathogen studies integrating genomic surveillance	Demonstrates biosurveillance expansion beyond pathogen presence toward resistance profiling; critical for AMR threat monitoring
Protozoan Parasites	Giardia spp., Cryptosporidium spp.	Low-to-moderate occurrence; primarily in studies with environmental health emphasis	Highlights feasibility of capturing protozoa in wastewater despite methodological challenges; relevant for diarrheal disease surveillance

Surveillance Technologies and Analytical Approaches

Twenty-seven studies employed molecular detection methods including RT-qPCR, metagenomic sequencing, and targeted PCR assays. Metagenomics provided the broadest detection spectrum and enabled discovery of emerging or unknown pathogens, although it required high computational and laboratory capacity. Wastewater concentration steps varied, with PEG precipitation and ultrafiltration being the most common.

Analytical innovations reported in the reviewed literature included:

- portable microfluidic PCR platforms for rapid field deployment
- sequencing-based pathogen discovery pipelines for variant tracking
- predictive modeling linking wastewater viral load to hospitalization trends
- automated sampling devices integrated into sewage networks for continuous detection

Linkages to Health Emergency Preparedness and Response

Evidence across studies demonstrated the value of multi-pathogen surveillance in:

Table 4: Linkages Between Multi-Pathogen Wastewater Surveillance and Health Emergency Preparedness and Response

Preparedness & Response Function	Evidence from Included Studies	Key Contribution of Multi-Pathogen Wastewater Surveillance
Outbreak Early Warning	Wastewater surveillance detected pathogen circulation 1–4 weeks earlier than conventional clinical reporting systems across multiple settings.	Facilitated proactive response measures by enabling authorities to anticipate case surges before symptomatic case detection.
Resource Allocation	Wastewater signals were used to geographically prioritize deployment of diagnostic test kits, vaccination outreach programs, and preventive interventions.	Supported optimized distribution of scarce public health resources during emergencies.

Variant and Mutation Tracking	Genomic sequencing of wastewater samples enabled continuous tracking of evolving pathogen sub-lineages, including emerging variants of concern.	Allowed near real-time identification of mutational shifts critical for response planning and therapeutic guidance.
Border and Entry Monitoring	Surveillance conducted near airports, border posts, and quarantine facilities captured imported transmission risks and informed containment actions.	Strengthened international health regulation compliance and reduced risk of cross-border spread.
Public Health Communication	Combined wastewater and epidemiological alerts improved public risk communication strategies and guided tailored community sensitization campaigns.	Enhanced community engagement, awareness, and adherence to control measures during emergency conditions.

Comparative Implementation Challenges and Enablers in Multi-Pathogen Wastewater Surveillance

Table 5 shows both the key challenges and enabling factors affecting the implementation of multi-pathogen wastewater surveillance, with clear quantitative patterns emerging across studies. Among the challenges, limited laboratory capacity and lack of standardized protocols are the most frequently reported, each cited by 63% of studies. This reflects widespread constraints related to inadequate laboratory infrastructure, shortages of skilled personnel, and inconsistent sampling and analytical methods. Insufficient integration into national surveillance systems and policy adoption gaps are each reported by 47% of studies, indicating that wastewater surveillance is often not fully embedded in routine public health decision-making. Other important challenges, though not assigned specific percentages, include variability in sewage infrastructure, which affects data representativeness, as well as data interpretation difficulties linked to population size, flow rates, and environmental conditions. Governance and data-sharing constraints further limit timely access to and use of surveillance data across institutions.

In contrast, several enabling factors show relatively high levels of support across literature. Cross-sectoral coordination is the most prominent facilitator, reported by 72% of studies, underscoring the importance of collaboration between public health agencies, environmental laboratories, and municipal authorities. Technological innovation follows closely at 66%, reflecting the role of advanced molecular tools and sequencing methods in expanding pathogen detection and early-warning capacity. Data platforms and analytics are reported by 53% of studies, while capacity building is noted by 50%, highlighting the value of training and improved data management. Policy and regulatory support appear in 47% of studies, reinforcing the role of formal guidelines in institutionalizing surveillance. Lower but still meaningful contributions are seen for community engagement (44%) and emergency funding (38%).

Table 5: Comparative Implementation Challenges and Enablers in Multi-Pathogen Wastewater Surveillance

Aspect	Key Points	Studies Reporting (%)	Description / Impact
Implementation Challenges	Limited laboratory capacity	63%	Low-resource settings often lack adequate lab infrastructure and trained personnel for multi-pathogen detection.
	Variability in sewage infrastructure	Not specified	Heterogeneous sewer networks reduce representativeness and reliability of surveillance data.
	Lack of standardized protocols	63%	Inconsistent sampling, concentration, and detection methods limit comparability across studies and regions.
	Insufficient integration into national surveillance	47%	Wastewater data is frequently not incorporated into official public health decision-making.
	Data interpretation challenges	Not specified	Population size, flow rates, and environmental factors complicate quantitative interpretation.
	Governance & data-sharing constraints	Not specified	Limited coordination between institutions restricts timely access and cross-agency utilization.
	Policy adoption gaps	47%	Transitioning surveillance from research to routine emergency preparedness requires formal policy support.
Enablers/ Facilitators	Cross-sectoral coordination	72%	Partnerships between public health agencies, environmental labs, and municipalities improve sample access and data sharing.
	Technological innovation	66%	Multiplex assays, portable RT-qPCR, and metagenomic sequencing expand pathogen coverage and early-warning capability.
	Policy and regulatory support	47%	National or local guidelines enable formal integration of wastewater surveillance into emergency preparedness.
	Data platforms & analytics	53%	Real-time dashboards and predictive models enhance rapid decision-making and outbreak forecasting.
	Community engagement	44%	Acceptance of sampling activities ensures access to sites and public support for surveillance programs.
	Emergency funding	38%	Rapid mobilization of resources allows urgent deployment during outbreaks.
	Capacity building	50%	Training improves technical skills, quality control, and long-term sustainability of surveillance efforts.

IV. DISCUSSION AND CONCLUSION

Discussion

The identification of 32 eligible studies from an initial pool of 532 records shows both the rapid expansion and the uneven maturity of the MPWS evidence base. The high exclusion rate at full-text review, largely due to limited methodological detail and narrow single-pathogen focus, mirrors concern raised in earlier reviews that wastewater surveillance literature is often exploratory and heterogeneous in quality (Bivins *et al*., 2020; Medema *et al*., 2020). This finding suggests that, while interest in wastewater-based epidemiology has grown substantially particularly during the COVID-19 pandemic, robust multi-pathogen designs explicitly linked to emergency preparedness remain comparatively limited. Nevertheless, the diversity of study designs retained in this review, including long-term monitoring, outbreak investigations, and implementation-focused analyses, indicates a transition from proof-of-concept studies toward applied public health tools. Similar transitions have been described in the evolution of environmental surveillance for poliovirus, where early laboratory-driven studies eventually informed global eradication strategies (Asghar *et al*., 2014; World Health Organization, 2015). The present findings therefore suggest that MPWS is at an intermediate stage of institutionalization, with growing empirical support but incomplete standardization.

The geographic distribution of studies reveals a clear imbalance, with Europe and North America accounting for more than half of the literatures included, while Africa, Latin America, and Oceania remain underrepresented. This pattern aligns with previous global assessments of wastewater surveillance capacity, which emphasize that high-income regions dominate methodological innovation due to stronger laboratory infrastructure and research funding (Daughton, 2020; Sims and Kasprzyk-Hordern, 2020). Inconsistently, the underrepresented regions often face higher burdens of waterborne and emerging infectious diseases, making the evidence gap particularly concerning from a global health equity perspective. The inclusion of studies conducted in informal settlements and low-resource areas, however limited in number, demonstrates the adaptability of MPWS beyond centralized sewer systems. Prior work has shown that even non-sewered or fragmented sanitation environments can yield actionable surveillance data when sampling strategies are contextually adapted (Berchenko *et al*., 2017; Kilpatrick *et al*., 2022). The current findings reinforce call for greater investment in MPWS research in low- and middle-income countries (LMICs), not only to improve local preparedness but also to enhance global outbreak intelligence.

A central finding of this review is the predominance of integrated surveillance approaches, with nearly three-quarters of studies monitoring both viral and bacterial pathogens. This marks a significant departure from traditional wastewater surveillance models that focused on single pathogens, such as poliovirus or SARS-CoV-2 alone (Asghar *et al*., 2014; Medema *et al*., 2020). The expanded pathogen scope strengthens the utility of MPWS as a comprehensive biosurveillance platform capable of addressing multiple, concurrent public health threats. The dominance of SARS-CoV-2 across studies is expected, given its role in catalyzing global adoption of wastewater surveillance during the COVID-19 pandemic. Multiple studies have demonstrated strong correlations between wastewater viral loads and subsequent clinical case trends, validating its role as an early warning system (Peccia *et al*., 2020; Wu *et al*., 2021). Importantly, the continued inclusion of poliovirus, particularly in LMIC settings, underscores the sustained relevance of wastewater surveillance for eradication and elimination programs beyond pandemic contexts (World Health Organization, 2022).

The integration of antimicrobial resistance (AMR) determinants and protozoan parasites in a subset of studies further expands the conceptual scope of MPWS. Environmental AMR surveillance has been increasingly recognized as a critical complement to clinical surveillance, offering population-level insights into resistance dissemination (Hendriksen *et al*., 2019). Although fewer studies addressed protozoa, their inclusion demonstrates methodological feasibility and highlights opportunities to strengthen diarrheal disease preparedness, especially in flood-prone and sanitation-challenged settings.

The predominance of molecular methods, particularly RT-qPCR and metagenomic sequencing, reflects current best practices in wastewater surveillance. RT-qPCR remains the backbone of routine surveillance due to its sensitivity, specificity, and relative affordability, while metagenomics offers unparalleled breadth and discovery potential (Choi *et al*., 2018; Thompson *et al*., 2023). The reviewed studies confirm that metagenomics enables simultaneous detection of known and emerging pathogens, as well as AMR genes, thereby enhancing situational awareness during complex health emergencies. However, the literature consistently acknowledges trade-offs between technological sophistication and feasibility. High costs, computational demands, and bioinformatics expertise limit the scalability of metagenomics in many LMICs, reinforcing concerns about technological inequities (Sims and Kasprzyk-Hordern, 2020). Innovations such as portable microfluidic PCR platforms and automated sampling systems, highlighted in several studies, represent promising pathways toward more decentralized and rapid deployment during outbreaks. These developments align with broader trends toward point-of-need diagnostics and real-time environmental surveillance (Keshaviah *et al*., 2021).

One of the most significant contributions of MPWS identified in this review is its consistent role in outbreak early warning. Evidence that wastewater signals precede clinical reporting by one to four weeks corroborates findings from multiple COVID-19 and norovirus surveillance studies (Peccia *et al*., 2020; Ahmed *et al*., 2021). This temporal advantage is particularly valuable in settings where healthcare access is limited or diagnostic testing is delayed, enabling authorities to implement anticipatory interventions. Beyond early warning, the use of wastewater data to guide resource allocation, variant tracking, and border monitoring demonstrates its operational relevance across the preparedness–response continuum. The ability to detect pathogen variants and mutations through wastewater genomics has been shown to complement clinical sequencing, especially when individual testing rates decline (Crits-Christoph *et al*., 2021). Furthermore, the integration of wastewater indicators into public risk communication strategies supports more targeted and credible messaging, which is critical for maintaining public trust during emergencies (World Health Organization, 2023).

The comparative analysis of challenges and enablers reveals that implementation barriers are predominantly structural and institutional rather than conceptual. Limited laboratory capacity and lack of standardized protocols, each reported by nearly two-thirds of studies, echo longstanding critiques of environmental surveillance fragmentation (Bivins *et al*., 2020). Without harmonized methods, cross-regional comparability and data sharing remain constrained, limiting the global utility of MPWS. Equally important are governance-related challenges, including weak integration into national surveillance systems and policy adoption gaps. Similar issues have been documented in the scale-up of integrated disease surveillance and response frameworks, where data availability does not automatically translate into policy action (Nkengasong *et al*., 2018). In contrast, the most prominent enablers cross-sectoral coordination, technological innovation, and capacity building highlight that MPWS thrives where environmental, public health, and municipal actors collaborate within supportive policy environments. The moderate emphasis on community engagement underscores an often-overlooked dimension of wastewater surveillance. Although sampling is non-intrusive, public acceptance and trust remain essential, particularly in informal settlements and emergency contexts. Prior studies suggest that transparent communication about surveillance objectives and benefits enhances legitimacy and sustainability (Lodder and de Roda Husman, 2020).

In practice, sustained investment in laboratory capacity, workforce training, and data analytics platforms will determine the long-term viability of MPWS. As global health threats become increasingly complex and interconnected, the ability of wastewater surveillance to provide early, integrated, and population-level intelligence positions it as a critical pillar of resilient health systems.

V. Conclusion

This systematic review demonstrates that multi-pathogen wastewater surveillance (MPWS) is an effective and adaptable population-level biosurveillance tool with significant value for health emergency preparedness and response. Evidence from the 32 included studies shows that MPWS can simultaneously detect a wide range of viral, bacterial, protozoan, and antimicrobial resistance indicators, often providing earlier warning of pathogen circulation than conventional clinical surveillance systems. Its successful application across diverse settings including centralized wastewater treatment plants, informal settlements, and outbreak-specific mobile units highlights its flexibility and relevance across both high-resource and constrained environments.

However, the review also identifies persistent challenges that limit the routine institutionalization of MPWS, particularly inadequate laboratory capacity, lack of standardized protocols, and weak integration into national surveillance and policy frameworks. Addressing these barriers through sustained investment in infrastructure, workforce training, cross-sectoral collaboration, and regulatory support is critical. With deliberate policy commitment and methodological harmonization, MPWS has strong potential to strengthen early detection, guide timely public health action, and enhance the resilience of health systems against emerging and re-emerging infectious disease threats.

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