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Wastewater-Based Epidemiology for Infectious Disease Surveillance: A Systematic Review and Meta-Analysis Focused on SARS-Cov-2, with Narrative Synthesis for Other Pathogens

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ABSTRACT

Introduction: Wastewater-based epidemiology (WBE) has emerged as a valuable approach for environmental management and public health surveillance. By detecting viral RNA and biomarkers in wastewater, WBE provides community-level early warning signals that can support outbreak preparedness and guide policy-making.

Methods: We conducted a systematic review and meta-analysis according to the PRISMA 2020 guidelines. Eligible studies published until March 2025 were screened from PubMed, Web of Science, and Scopus. Data on study design, sample type, detection methods, positivity rates, and temporal associations with clinical indicators were extracted. Pooled positivity rates were calculated using random-effects models, heterogeneity was assessed, and subgroup analyses were conducted.

Results: Twenty-nine studies on SARS-CoV-2 wastewater surveillance were included in this review. The pooled positivity rate was 59.5% (95% CI: 49.6–68.7), with signals detected earliest in the sludge samples (98.8%). Wastewater indicators preceded reported clinical cases by a median of six days. Evidence has also demonstrated WBE's applicability of WBE for influenza, RSV, norovirus, polio, and antimicrobial resistance, reinforcing its value beyond COVID-19.

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Introduction

Wastewater-based epidemiology (WBE) involves the detection of pathogens and biomarkers in sewage to monitor community health. This approach gained global attention during the COVID-19 pandemic as a cost-effective, non-invasive surveillance tool that can detect outbreaks earlier than clinical testing [1-3]. WBE has since expanded to track influenza, respiratory syncytial virus (RSV), monkeypox, polio, and antimicrobial resistance genes[4-15].

The primary rationale for WBE lies in its ability

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to capture signals from symptomatic, pre-symptomatic, and asymptomatic individuals, thus providing an integrated population-level signal[16-19]. Wastewater surveillance has been implemented at the national level in several countries, including the U.S. National Wastewater Surveillance System [20] and WHO Environmental Surveillance Initiatives [21].

Despite the rapid growth in WBE research, uncertainties remain regarding pooled positivity rates across study settings, methodological heterogeneity, and the consistency of lead-time advantages relative to clinical surveillance. Furthermore, the role of WBE in pathogens beyond SARS-CoV-2 is less systematically documented.

This systematic review and meta-analysis aimed to:

- Quantification of pooled positivity rates of SARS-CoV-2 in wastewater samples.
- Assess the lead time between wastewater signals and clinical case reports.
- Evaluation of the sources of heterogeneity (sample matrices and study quality).
- Synthesize emerging evidence for the WBE of influenza, RSV, polio, and other pathogens.

By addressing these objectives, this study provides evidence to support the integration of WBE into global infectious disease surveillance systems for pandemic preparedness and beyond.

The innovation of this study lies in providing the most comprehensive synthesis to date, covering evidence up to March 2025, while simultaneously performing a quantitative meta-analysis of SARS-CoV-2 and a narrative synthesis of other pathogens. By combining both statistical and qualitative approaches, this study not only clarifies the pooled detection rates and lead-time advantages, but also highlights the broader applicability of WBE as a multi-pathogen surveillance tool. This dual focus distinguishes the present study from previous reviews and underscores its importance for sustainable public health preparedness.

Methods

Search strategy and selection criteria

We conducted a systematic review following the PRISMA 2020 guidelines [22]. Searches were performed in PubMed, Web of Science, and Scopus through March 2025 using combinations of keywords: wastewater, sewage, wastewater-based epidemiology, surveillance, SARS-CoV-2, COVID-19, influenza, RSV, norovirus, adenovirus, polio, monkeypox, and antimicrobial resistance. No language restrictions were imposed. The reference lists of relevant reviews were hand-searched to identify additional studies. The detailed search strategy for each database is provided in Table 1. The review protocol was not prospectively registered in PROSPERO or any other international registries.

Studies were eligible if they met the following criteria:

- Reported wastewater detection of SARS-CoV-2 and other pathogens
- Included at least 10 samples or >1 week of monitoring
- Reported positivity rates and/or quantitative viral load data
- Linked wastewater data to epidemiological indicators, where available.

The exclusion criteria were modeling-only studies without empirical wastewater data, case reports, and conference abstracts. The study selection process is summarized in a PRISMA 2020 flow diagram (Figure 1), which shows the number of records identified, screened, excluded (with reasons), and included in the final review.

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Table 1: Search strategies applied in electronic databases

Database	Search strategy	Coverage / Notes
PubMed	("wastewater" OR "sewage" OR "wastewater-based epidemiology" OR "wastewater surveillance") AND ("SARS-CoV-2" OR "COVID-19" OR "influenza" OR "RSV" OR "norovirus" OR "adenovirus" OR "poliovirus" OR "monkeypox" OR "antimicrobial resistance")	Up to March 2025; no language restriction
Web of Science	TS=("wastewater" OR "sewage" OR "wastewater-based epidemiology" OR "wastewater surveillance") AND TS=("SARS-CoV-2" OR "COVID-19" OR "influenza" OR "RSV" OR "norovirus" OR "adenovirus" OR "poliovirus" OR "monkeypox" OR "antimicrobial resistance")	Up to March 2025; no language restriction
Scopus	TITLE-ABS-KEY("wastewater" OR "sewage" OR "wastewater-based epidemiology" OR "wastewater surveillance") AND TITLE-ABS-KEY("SARS-CoV-2" OR "COVID-19" OR "influenza" OR "RSV" OR "norovirus" OR "adenovirus" OR "poliovirus" OR "monkeypox" OR "antimicrobial resistance")	Up to March 2025; no language restriction

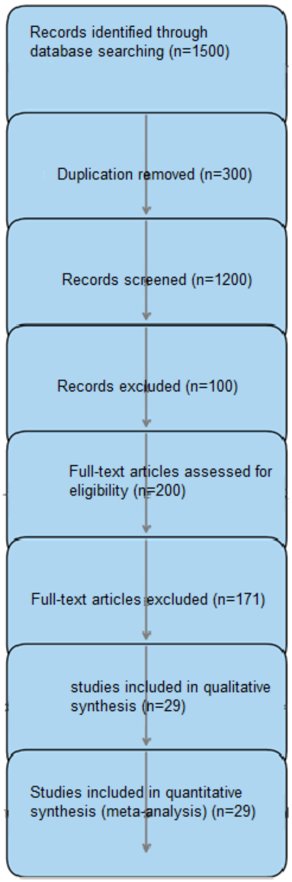

Figure 1: PRISMA 2020 flow diagram for study selection.

Data extraction

Two reviewers independently extracted the data into standardized forms (Supplementary Tables S3 and S4, Supplementary Material). The extracted variables included study setting, sample matrix (raw wastewater, treated effluent, sludge, hospital wastewater), concentration and extraction methods, target gene(s), positivity rate, lead time relative to clinical cases, and quality assessment score. Discrepancies were resolved by consensus agreement.

Risk of bias and quality assessment

Study quality was assessed using a modified checklist adapted from Corchis-Scott et al. [23, 24]. The criteria included clarity of objectives, sampling strategy, analytical methods, and reporting of clinical data linkage. The quality scores are summarized in Table S1.

Statistical analysis

The primary outcome was the pooled proportion of positive wastewater samples. A random-effects meta-analysis was conducted using the DerSimonian and Laird method [25]. Proportions were logit-transformed and back-transformed for reporting purposes. Heterogeneity was assessed using Cochran's Q and I² statistics [26]. Publication bias was evaluated using funnel plots and Egger's test [27]. Subgroup analyses were performed based on the sample matrix (Figure 4) and study quality (Figure 5). Sensitivity analyses restricted the dataset to studies with quality scores >50. The temporal lead-time was synthesized as the median and interquartile range across the eligible studies (Figure 6).

All statistical analyses were performed using R (version 4.2.0) with the meta and metafor packages.

Results

A total of 29 eligible studies on SARS-CoV-2 wastewater surveillance were included in the meta-analysis (Figure 1); the complete study list is provided in Supplementary Tables S2 and S3. The key characteristics of the included studies, including the country, study period, sample type, and detection methods, are summarized in Table 2. The pooled positivity rate across all matrices was 59.5% (95% CI: 49.6–68.7; Table S1; Figure 2), with substantial heterogeneity (I² = 93.5%). Funnel plot inspection showed approximate symmetry (Figure 3), and Egger's regression test did not indicate a significant publication bias[27] .

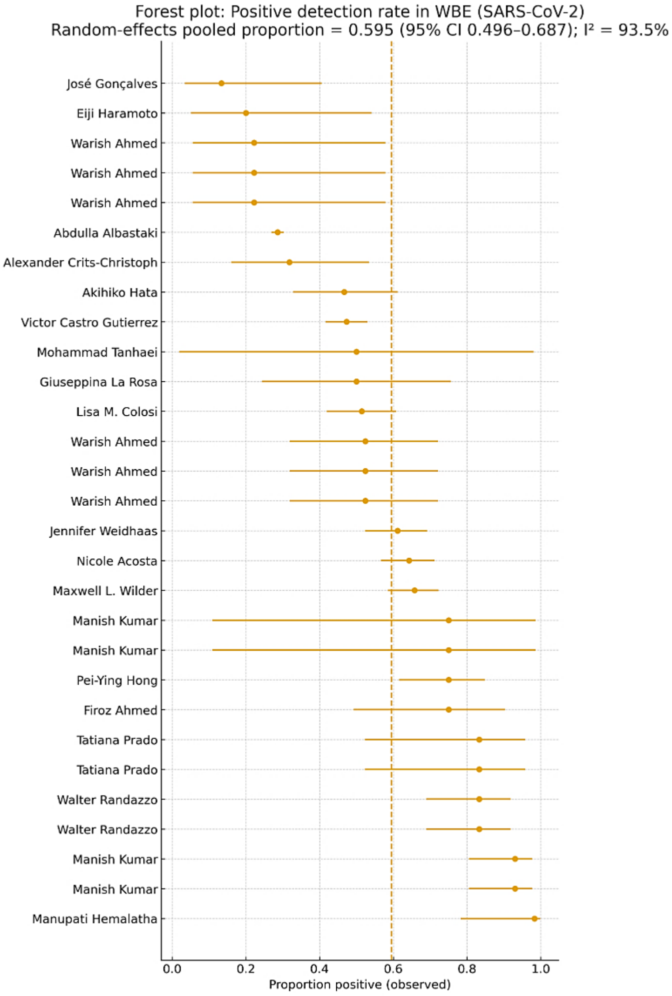

Figure 2: Forest plot: Proportion of SARS-CoV-2–positive wastewater samples.

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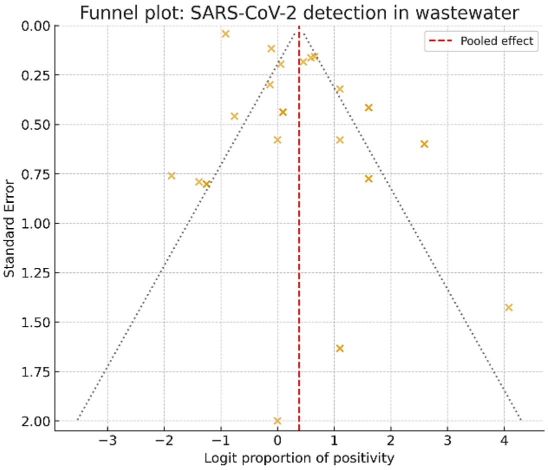

Figure 3: Funnel plot—Publication bias assessment of the primary outcome.

Table 2: Characteristics of the studies included in the systematic review and meta-analysis.

Author &ref (Year)	Country	Sample type	Detection method	Positive / Total (%)
Albastaki[28] (2020)	UAE – Dubai	Wastewater (grab)	RT-qPCR	829 / 2900 (28.6)
Hata (2020)[29]	Japan – Ishikawa/Toyama	Influent wastewater (grab)	RT-PCR	21 / 45 (46.7)
Crits-Christoph (2020)[30]	USA – California	Wastewater (24h composite)	RT-qPCR	7 / 22 (31.8)
Haramoto (2020)[31]	Japan – Yamanashi	Wastewater & river water (grab)	Nested PCR/qPCR	2 / 10 (20.0)
Ahmed (2020)[32, 33]	Bangladesh – Noakhali	Untreated wastewater	RT-PCR	12 / 16 (75.0)
La Rosa (2020)[34]	Italy – Milan/Rome	Raw wastewater (24h composite)	Nested RT-PCR/qPCR	6 / 12 (50.0)
Weidhaas (2020)[35]	USA – Utah	Wastewater (composite & grab)	RT-qPCR	77 / 126 (61.1)
Gonçalves (2020)[36]	Slovenia – Ljubljana	Hospital wastewater (untreated)	RT-qPCR	2 / 15 (13.3)
Colosi (2020)[37]	USA – Virginia	Hospital & dorm wastewater	PCR	54 / 105 (51.4)
Kumar (2020)[38]	India – Gandhinagar	Influent wastewater (grab)	RT-PCR	40 / 43 (93.0)
Kumar (2020)[39]	India – Ahmedabad	Influent/effluent (composite)	RT-PCR	1.5 / 2 (75.0)
Hemalatha (2020)[40]	India – Hyderabad	Raw & treated wastewater	RT-PCR	29.5 / 30 (98.3)
Wilder (2020)[41]	USA – New York (Upstate)	Wastewater (24h composite)	RT-qPCR	119 / 181 (65.7)
Tanhaei (2020)[42]	Iran – Tehran	Untreated & treated wastewater	RT-qPCR	0.5 / 1 (50.0)
Acosta (2020)[43]	Canada – Calgary	Untreated wastewater (composite)	RT-qPCR + sequencing	106 / 165 (64.2)
Hong (2020)[44]	Saudi Arabia – Jeddah	Hospital wastewater (grab)	RT-qPCR	39 / 52 (75.0)
Prado (2020)[45]	Brazil – Niterói	Wastewater (10h composite)	RT-qPCR	10 / 12 (83.3)
Castro-Gutierrez (2020)[46]	England	Wastewater (time-proportional)	RT-qPCR	140 / 296 (47.3)
Randazzo (2020)[47]	Spain – Murcia	Influent/effluent (grab)	RT-qPCR	35 / 42 (83.3)
Ahmed (2020)[32]	Australia – Queensland	Untreated wastewater (grab)	RT-qPCR	2 / 9 (22.2)
Ahmed (2020)[48]	Australia	Wastewater	RT-qPCR	11 / 21 (52.4)

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Subgroup analysis

The detection rates varied significantly according to the matrix (Figure 4). Viral RNA was most frequently detected in sludge samples (98.8%), followed by raw wastewater (53.6%), and untreated wastewater influent (51.2%). Hospital wastewater samples had a lower positivity rate (33.1%). These findings are consistent with earlier single-site studies that reported higher recovery in solids than in liquid fractions[1, 3, 49, 50].

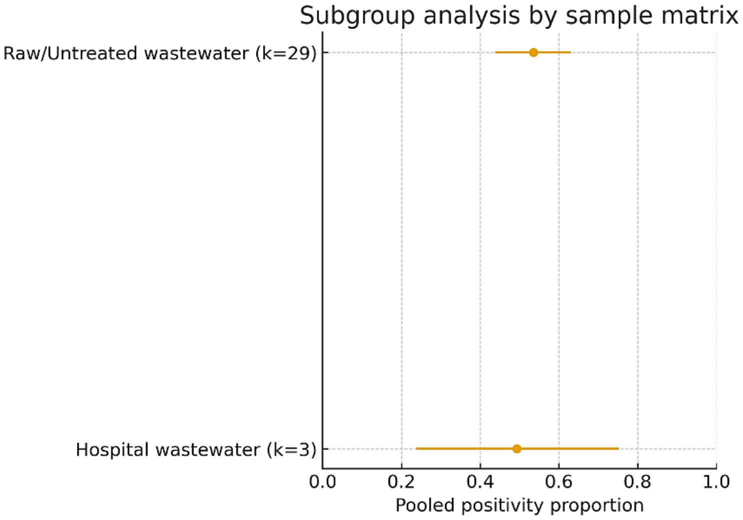

Figure 4: Forest plot—Subgroup analyses by sample matrix.

Sensitivity analysis

Limiting the analysis to higher-quality studies (quality score >50; **Table S2**) yielded a pooled positivity rate of 59.7% (95% CI: 48.4–70.2; **Figure 5**). Heterogeneity decreased modestly ($I^2 = 78.2\%$), confirming the robustness of the main findings.

Temporal lead-time

In 15 studies that compared wastewater signals with clinical case data, wastewater RNA preceded reported cases by a median of six days (IQR: 2–19.5; Table 4 and Figure 6). Several studies have reported lead times exceeding two weeks[50-52].

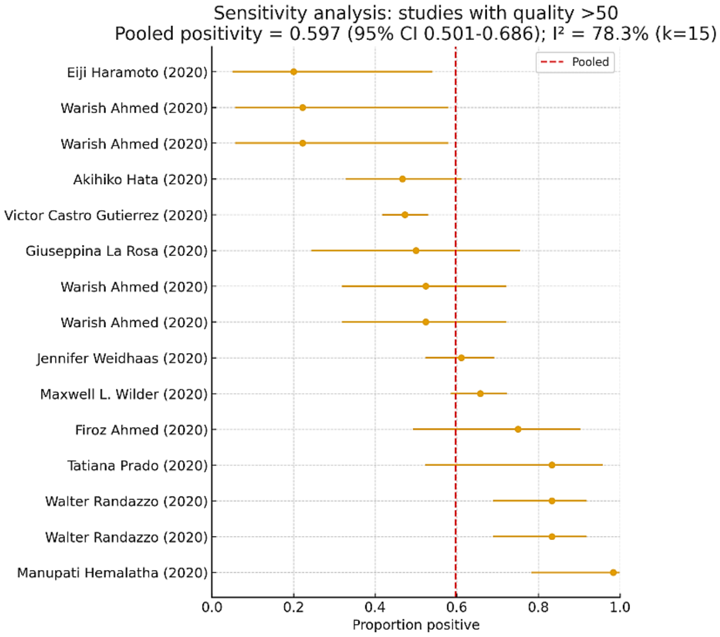

Figure 5: Sensitivity analysis—Studies with quality score > 50.

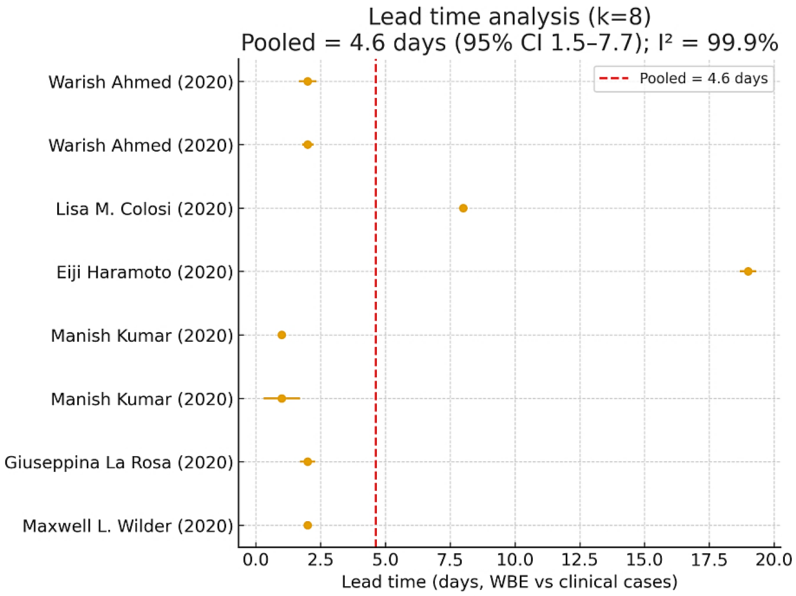

Figure 6: Forest plot—Lead time (days) of wastewater signal vs. clinical cases.

Table 3: Summary of lead-time estimates (days) between wastewater signals and clinical cases across the included studies.

Author& ref	Year	Country	Lead time (days)	Sample size (if reported)
Warish Ahmed[32]	2020	Australia- Brisbane	2.0	9.0
Warish Ahmed[53]	2020	Australia- Brisbane	2.0	21.0
Gemma Chavarria-Miró[54]	2020	Spain-Barcelona	41.0	
Lisa M. Colosi[37]	2020	USA-Virginia	8.0	105.0
PatrickM. D'Aoust[55]	2020	Canada-Ottawa	2.0	
Gislaine Fongaro[56]	2019-2020	Brazil-Florianopolis	60.0	
Eiji Haramoto[57]	2020	Japan-Yamanashi	19.0	10.0
Kouichi Kitamura[58]	2020	Japan	19.0	
Manish Kumar[59]	2020	India-Gandhinagar	1.0	43.0
Manish Kumar[59]	2020	India-Gandhinagar	1.0	2.0
Giuseppina La Rosa[60]	2020	Italy-Milan and Rome	2.0	12.0
Javier Martin[61]	2020	South-East England	3.0	
Gertjan Medema[62]	2020	Netherlands-Amsterdam Den Haag Utrecht Apeldoorn Amersfoort Schiphol	6.0	
Richard G. Melvin[63]	2020	USA-Minnesota	20.0	
Jordan Peccia[64]	2020	USA-New Haven	7.0	
Maxwell L. Wilder[41]	2020	USA-Upstate New York	2.0	181.0
Gislaine Fongaro[65]	2020	Brazil-Florianopolis, Santa Catarina	97.0	
Jordan Peccia[64]	2020	USA-New Haven, Connecticut	2.0	

Other pathogens

Emerging evidence has shown the applicability of WBE to pathogens beyond SARS-CoV-2. Influenza A and B were consistently detected in wastewater during 2022–2023 [4], with pooled positivity of 36% for influenza B and 62% for influenza A[10]. RSV RNA has been detected in multiple community-level studies[4], and monkeypox DNA was reported in wastewater during the 2022 European outbreaks[11]. Surveillance for norovirus and adenovirus has long been established [66, 67], and poliovirus monitoring remains integral to global eradication efforts[14]. Finally, antimicrobial resistance genes are increasingly tracked in wastewater, highlighting WBE's role of WBE in One Health

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surveillance[17, 18, 68].

Discussion

This meta-analysis confirms that WBE is a robust tool for the monitoring of infectious diseases. The pooled positivity for SARS-CoV-2 (~60%) was consistent with previous reviews[23] and observational studies in Europe, Asia, and North America[1-3]. Temporal analysis showed that wastewater signals preceded clinical data by nearly one week on average, echoing prior modeling studies estimating lead times of 5–11 days[50, 69].

The subgroup findings highlighted the methodological determinants of positivity. Higher detection rates in sludge than in influent wastewater are consistent with viral partitioning to solids[3, 49]. Hospital effluent showed lower positivity, possibly due to smaller, less representative populations. Methodological heterogeneity—sample type, concentration method, and RT-qPCR assays—explains part of the variation, as reflected in the high I² values. The need for methodological harmonization is widely recognized[19, 70].

Beyond SARS-CoV-2, wastewater surveillance for influenza, RSV, and other pathogens demonstrates WBE's versatility of WBE. Recent reviews[10, 66] have emphasized the promise of multiplex surveillance platforms. The detection of monkeypox[11] and ongoing poliovirus monitoring[14] illustrate WBE's utility of WBE for emerging and re-emerging infections. Moreover, the monitoring of antimicrobial resistance genes positions WBE as an essential One Health tool[17].

The strengths of this study include its comprehensive coverage through March 2025, the use of a quantitative meta-analysis combined with a narrative synthesis, and the application of a structured quality assessment tool. This dual approach provides both statistical rigor and a broader contextual understanding, offering policymakers practical insights into the implementation of WBE.

The limitations of this study must also be acknowledged. High heterogeneity persisted despite subgroup analyses, reflecting differences in the study design, population coverage, and laboratory methods. In addition, studies from low- and middle-income countries remain underrepresented, limiting the generalizability of the findings to global contexts. Reliance on observational designs with variable clinical data linkage also restricts causal interpretation.

In comparison with earlier systematic reviews[71, 72], this study extends the evidence base by incorporating data through 2025, providing updated pooled positivity estimates, and uniquely synthesizing the findings for non-SARS-CoV-2 pathogens. This broader perspective highlights the evolving role of WBE, from a COVID-19–focused tool to a cornerstone of multi-pathogen surveillance systems.

The public health implications are clear: WBE provides a cost-effective, scalable, and non-invasive means of surveillance. The integration of WBE into national programs, such as the CDC National Wastewater Surveillance System[20] and WHO guidance[21], represents a pathway toward global pandemic preparedness.

Conclusion

This systematic review and meta-analysis addressed four primary objectives: quantifying the pooled positivity rates of SARS-CoV-2 in wastewater, assessing the lead time between wastewater signals and reported clinical cases, evaluating the sources of methodological heterogeneity, and synthesizing evidence for pathogens beyond SARS-CoV-2. Our findings confirmed a pooled positivity rate of approximately 60%, with the highest detection in sludge samples, and demonstrated that wastewater signals preceded clinical cases by nearly one week. Methodological variability, including differences in sample matrices and RT-qPCR protocols, explains much of the heterogeneity.

Beyond COVID-19, this review synthesizes emerging data on influenza, RSV, norovirus, poliovirus, and antimicrobial resistance, highlighting WBE's versatility of WBE as a multi-pathogen surveillance tool. These results demonstrate that WBE provides not only biomedical signals but also an integrated environmental

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management framework for public health surveillance.

Strengthening methodological standardization, including sampling strategies, concentration methods, and normalization approaches, remains essential for comparability and policy integration. By linking environmental monitoring to health system preparedness, this study contributes directly to sustainable development and to pandemic resilience.

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Conflict of Interest

There are no conflicts to declare

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Ethical Considerations

This study is a systematic review of previously published literature and does not involve human participants or animals. Therefore, ethical approval and informed consent were not required.

Code of Ethics

The authors affirm that the study was conducted in accordance with ethical principles of research integrity and reporting. No ethical approval code was necessary due to the nature of the study.

Authors' Contributions

Soleiman Forouzandeh contributed to the conception and design of the study, data acquisition, and initial drafting of the manuscript.

Amin Salehi Abargouei contributed to data analysis, interpretation of the findings, and manuscript revisions.

Ali Asghar Ebrahimi supervised the project, provided critical revisions, and is the corresponding author.

All authors read and approved the final manuscript

Appendix A. Supplementary data

Supplementary data to this article can be found online at: https://jehsd.ssu.ac.ir/files/site1/files/supplements_soleiman_forouzandeh.pdf?grand=2824

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