Environmental Science and Ecotechnology 28 (2025) 100626

Contents lists available at ScienceDirect

Environmental Science and Ecotechnology

journal homepage: www.journals.elsevier.com/environmental-science-and-ecotechnology/

Review

Immunoproteomics for wastewater-based health surveillance: A review

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ARTICLE INFO

Article history:
Received 2 April 2025
Received in revised form
30 September 2025
Accepted 1 October 2025

Keywords:
Wastewater-based epidemiology
Immunoproteomics
Mass spectrometry

ABSTRACT

Wastewater-based epidemiology (WBE) offers a unique window into the health and habits of communities through the analysis of pollutants and biomarkers in sewage. Traditionally focused on small molecules, such as pharmaceuticals and illegal drugs, recent advances in environmental proteomics have expanded WBE to include large biomolecules such as proteins. Notably, novel sampling methods using polymeric probes and high-resolution mass spectrometry have facilitated the detection of human and animal proteins, both soluble and in particulate material, linking them to specific populations and industrial activities. An immunological dimension to this approach is fundamental to include the recognition of host immunoglobulins, immune-response proteins, and pathogen antigens in wastewater, potentially serving as indicators of community immune status, infection prevalence, and vaccination coverage. This review consolidates the latest advancements in environmental proteomics as applied to WBE, emphasizing an immunological perspective as a comprehensive tool for assessing population health and environmental conditions to bridge environmental monitoring, public health, and clinical diagnostics.

1. Introduction

Wastewater-based epidemiology (WBE)—also known as sewage or wastewater epidemiology—has emerged as a valuable tool for population-level health surveillance, providing a picture of community-wide activities, lifestyle, and health status through the analysis of pollutants and biomarkers in sewage [1–3]. This approach uses sewage collected from wastewater systems, such as plants and sewers, as an anonymized composite of excreta and other community residues. It captures traces of small molecules, such as pharmaceutical metabolites and illicit drugs; microbial agents, including viruses; and, increasingly, macromolecules such as proteins. These components can be analyzed to evaluate the overall health status of the population served by the system [4].

The early success of WBE was primarily based on detecting small organic compounds [1] and genetic material from pathogens, most notably severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the COVID-19 pandemic [5,6]. More recently, interest has grown in the role of proteins in WBE. Pioneering studies have identified a variety of human-derived proteins in wastewater, such as uromodulin, α-amylase, and S100A8—all previously proposed as health markers [7–9]. In addition to human proteins, additional sources, such as livestock residues and microbial byproducts, support wastewater's complex and multi-organism proteomic landscape, underscoring a larger environmental dimension of this field.

An immunological perspective adds further depth by targeting

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https://doi.org/10.1016/j.ese.2025.100626

human antibodies, including total immunoglobulins and disease-specific antibodies [10], alongside immune-related proteins, such as defensins, cytokines, chemokines, and complement factors [9,11], as well as pathogen antigens [12,13]. These markers offer detailed insights into community immune status, infection prevalence, and vaccination coverage. For example, changes in immunoglobulin levels may serve as early indicators of outbreaks, while shifts in antigenic protein levels could signal the evolution of infection dynamics.

This review consolidates current knowledge on environmental proteomics in the context of WBE, with a particular focus on its immunological dimension. It highlights how integrating immune-related protein analysis can enhance WBE's capacity for comprehensive public health and environmental monitoring.

2. Bibliographic search methods

To position this article within the rapidly evolving WBE literature, we conducted a purposive narrative search of PubMed, Scopus, and Web of Science databases up to June 30, 2025. Given the relatively recent emergence of environmental proteomics, no earlier date limit was applied, and a small number of pre-2020 publications were included due to their seminal contributions, such as early uses of immunoaffinity, mass spectrometry (MS), biosensor platforms for viral antigen detection, and proteomic methods for environmental monitoring.

The core Boolean expression, adapted to the syntax of each database, combined wastewater terminology with protein-focused analytics: (wastewater OR sewage) AND (proteomics OR LC-MS OR LC-MS/MS OR immunoassay* OR biosensor* OR antigen OR antibody) AND (epidemiology* OR biomarker* OR population OR surveillance).

Study selection followed a relevance-based, rather than exhaustive, logic appropriate for narrative synthesis. We retained reports that (i) analyzed wastewater, sludge, or sewer samples; (ii) used MS, immunoextraction, or biosensor platforms; and (iii) yielded insights into public health surveillance, pathogen detection, or antimicrobial resistance. We excluded non-English publications and conference abstracts without accompanying full papers.

Data extraction focused on matrix types, sample processing workflows, analytical platforms, target proteins, detection limits, and epidemiological contexts. The selected studies were then organized into four thematic strands, reflecting the evolution of the field: (i) untargeted discovery proteomics, (ii) targeted immunoproteomics for specific antigens or host proteins, (iii) rapid biosensor technologies, and (iv) integrative multiomic applications.

3. Wastewater proteomics

Environmental proteomics is an emerging field that employs proteomic technologies to analyze complex biological samples, such as wastewater, to gain insights into environmental and public health issues. This approach is particularly relevant to WBE, as the analysis of proteins in wastewater can aid in monitoring community health and environmental dynamics [14,15].

Although most proteomic studies of wastewater treatment plants (WWTPs) have focused on microbial consortia involved in biological treatment processes [16], pioneering investigations have shown that proteins from diverse sources—including human, animal, and microbial—can be detected in influent and effluent as well as in dissolved and particulate phases [8,9]. These findings emphasize the need for tailored analytical strategies to characterize such complex matrices. Standardized workflows are essential due to the low abundance of target proteins and interfering substances, as well as the dominance of high-concentration compounds. As a result, wastewater proteomics typically involves processing large sample volumes, followed by filtration and concentration steps [7,9,10,17]. Additional fractionation procedures, such as gel electrophoresis, may be required to enrich low-abundance proteins and reduce interference [7,9]. Proteins are commonly digested with trypsin into shorter peptides, which are better suited for downstream analysis via MS [14]. Two MS-based approaches predominate: (i) untargeted proteomics, in which all detectable peptides are analyzed in data-dependent or data-independent acquisition modes and matched to databases (shotgun proteomics), and (ii) targeted proteomics, which focuses on predefined proteotypic peptides, which are unique to proteins of interest and optimized for MS detection.

To address the challenge of handling large wastewater volumes, researchers have used polymer probes combined with untargeted proteomics for large-scale profiling across WWTP water streams [8,9]. This approach has detected hundreds of proteins from bacterial, plant, animal, and human sources, with variations across sampling sites and treatment steps. Notably, human proteins have been found to be abundant in influent but largely absent from effluent. Both common proteins (e.g., albumins and keratins) and low-abundance markers (e.g., S100A8, uromodulin, and defensins) have been identified, many of which are potential disease biomarkers.

However, the polymer-based method requires a prolonged exposure time (immersion) and may introduce bias due to polymer affinity or biofilm formation. Therefore, researchers have also employed automated sample collection systems at WWTP inlets, followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Shotgun proteomics has been utilized to analyze both soluble and particulate fractions across municipalities with varying population sizes and industrial activities, with samples collected at multiple time points [7]. These analyses have yielded comprehensive profiles of proteins from prokaryotic (bacteria and viruses) and eukaryotic (plants, birds, mammals, and humans) sources. Human excreta and livestock residues have emerged as dominant contributors. Soluble fractions have been enriched in Chordata-derived proteins, while particulate fractions have been predominantly bacterial. These studies underscore the importance of fraction-specific analyses and establish a strong foundation for expanding wastewater proteomics into public health surveillance.

Polymerase chain reaction (PCR)–based methods are commonly used for detecting viral genomes in wastewater (e.g., poliovirus [18], hepatitis B virus [19], and SARS-CoV-2 [20]). However, they require specialized personnel and infrastructure as well as optimized recovery protocols. In contrast, LC-MS/MS offers several potential advantages, including shorter run times [21] and greater sensitivity [22], as well as the ability to simultaneously detect pathogens and host proteins in both untargeted and targeted workflows. This enables earlier and more robust surveillance. For example, untargeted MS assays have identified SARS-CoV-2 proteins in wastewater up to six days ahead of reported case data [12]. Peptides have been detected from structural proteins (membrane, spike, and nucleocapsid proteins), transmembrane proteins (ORF3a, ORF7a, ORF8), and, notably, the nonstructural protein pp1ab—likely excreted via urine or feces—suggesting its potential as a COVID-19 biomarker.

Another study, using data-independent acquisition (DIA) mode LC-MS/MS, identified several human proteins associated with infectious diseases, including immunoglobulins, cytokines, chemokines, complement factors, and antimicrobial proteins, many of which have correlated with local COVID-19 case counts [11].

The targeting of both SARS-CoV-2 and C-reactive protein (CRP)

in wastewater has demonstrated how MS can provide early insights into infection and immune activation at the community level [17]. This dual-target strategy supports the value of MS for early warning, offering advantages over PCR by allowing simultaneous monitoring of viral presence and host response.

These proof-of-concept studies have also highlighted the need for enhanced analytical precision and reproducibility, thereby advancing wastewater proteomics toward scalable applications in public health. To facilitate broader adoption, standardized operating parameters are essential. Therefore, Table 1 condenses the settings reported in recent wastewater proteomics studies.

In summary, untargeted workflows support broad protein discovery without prior assumptions [7–9,11,12], while targeted approaches have proven critical for immunosurveillance [17]. Importantly, LC-MS/MS enables hybrid workflows for the simultaneous screening of small and large molecules, thereby overcoming historical divides in analytical approaches [14]. This integration is particularly valuable in WBE, as it offers a multidimensional perspective on community health.

Despite these advances, the application of immunoproteomics in WBE still faces workflow challenges, including sample complexity, matrix interferences, lack of standardization, and limited target-specific reagents. Table 2 summarizes the key obstacles and proposed solutions aimed at improving the reproducibility, reliability, and real-time applicability of proteomics in WBE.

4. The immunological dimension

In parallel with proteomics, immunosurveillance in wastewater focuses on detecting immunological markers, such as antibodies, cytokines, and immune-responsive proteins, providing deeper insight into population-level health dynamics. While proteomics detects pathogen-derived proteins and general human immunoglobulins in wastewater [9], immunological assays reveal pathogen-specific antibodies, offering more precise information about exposure and immune status. Recent studies have shown that combining proteomic and immunological assays enables both pathogens tracking and the assessment of community-wide immune protection. Proteomic workflows, including MS, have consistently identified substantial amounts of human immunoglobulin chain peptides in wastewater [7], indicating the presence of pathogen-specific circulating antibodies. Concurrently, MS and immunological techniques, such as western blotting and enzyme-linked immunosorbent assay (ELISA), have confirmed the presence of intact, functional antibodies (IgG, IgA) in wastewater samples from both neighborhood-scale and centralized facilities. These antibodies have demonstrated binding affinity for pathogens such as SARS-CoV-2 and influenza A [10]. By measuring pathogen-specific antibodies in wastewater, public health authorities can estimate the extent to which a population has achieved or maintained protective immunity. This is especially valuable when monitoring vaccination campaigns or emerging infectious threats.

In addition to antibodies, proteins excreted in feces and urine during infection present new opportunities for noninvasive

Table 1
Summary of workflow parameters supporting reliable wastewater proteomics.

Workflow step	Key parameter	Recommended setting	Rationale	Reference
Sample collection	Frequency	Daily/weekly/seasonal, as per study design	Aligns temporal resolution with surveillance goal	[7], [9], [17]
	Collection mode	Autosampler or polymeric sorbent probe	Representative influent load	[7], [9], [17]
	Volume	30–100 mL	Balances protein yield with centrifuge capacity	[7], [9], [17]
	Optional ultracentrifugation	100,000×*g*, 4 °C, 60 min	Separates soluble/particulate fractions	[7]
	Storage	−80 to −20 °C	Minimizes proteolysis before extraction	[11], [17]
Preprocessing and concentration	Debris removal	15,000×g, 4 °C, 15 min	Clarifies sample for next steps	[7], [11]
	Filtration	0.2 μm filter	Removes colloids	[7]
	Option A: ultrafiltration	10 kDa spin filter	Retains proteins; detergent compatible	[7], [17]
	Option B: solid-phase extraction	pH adjustment, C8 cartridge, 50% ACN/0.1% TFA elution	Enrichment of soluble proteins	[11]
	Option C: precipitation	1:1 sample/cold acetone, o/n, −20 °C	Low-cost total protein capture	[12]
Protein extraction (particulate)	Lysis buffer	2% SDS, 50 mM DTT, 75 mM Tris-HCl, pH 8	Denatures and reduces	[9]
	Heat/disruption	95 °C, 30 min; bead-beating	Maximizes release from sludge	[9]
Prefractionation (optional)	Additional concentration step	Electrophoresis, Coomassie staining, band excision	Full sample concentration	[7], [9]
Reduction and alkylation	Reagents	10 mM DTT, 55 mM iodoacetamide	Standard protein denaturation	[7]
Enzymatic digestion	Enzyme-to-protein ratio	Trypsin 1:50 (w/w)	Near-complete cleavage	[11], [17]
	Time/temperature	8 h (in-gel digestion) or o/n, 37 °C	Complete digestion	[9], [17]
Enrichment (optional)	Immunocapture	1 h, RT with antibody-magnetic beads	Boosts sensitivity for target panels	[17]
Discovery LC-MS/MS	Column/flow	100 μm × 150 mm C18, 400 nL min^{-1}	Nano-LC for sensitivity	[9]
	Gradient	60 min, 0–35% ACN/0.1% FA (phase B)	Deep proteome coverage	[9]
	Mass analyzer	Orbitrap; top 10 DDA at 60,000 or 4 m/z-window DIA at 30,000	High-resolution identification	[9], [11]
Targeted LC-MS/MS	Column/flow	2.1 mm × 30–50 mm C18, 0.3 mL min^{-1}	Ultra-high-performance-LC	[17]
	Gradient	0–5 min, 0–30% B; to 90% B, 10.5 min; hold 90% B to 16.8 min; 2 min re-equilibration	Fast cycle, high throughput	[17]
	Mass analyzer	Triple-quadrupole; positive-electrospray ionization, scheduled MRM with three transitions per peptide	Quantitative MRM	[17]

Abbreviations: ACN, acetonitrile; DDA, data-dependent acquisition; DIA, data-independent acquisition; DTT, dithiothreitol; FA, formic acid; LC, liquid chromatography; MRM, multiple-reaction monitoring; MS/MS, tandem mass spectrometry; o/n, overnight; RT, room temperature; SDS, sodium dodecyl sulphate; TFA, trifluoroacetic acid.

Table 2
Current challenges and emerging solutions for advancing wastewater immunoproteomics.

Challenge	Details	Potential solutions
Method standardization	Proteomic protocols vary significantly in terms of sample preparation (e.g., digestion, enrichment), instrument settings, and data analysis pipelines. Lack of standardization could affect reproducibility and comparison across studies.	- Development of shared protocols (e.g., by initiatives such as the Human Proteome Organization). - Use of isotopically labeled peptide standards for quantification.
Sample complexity and low abundance of targets	Viral and immune proteins are present at very low concentrations among interfering substances in sewage.	- Application of sample concentration strategies. - Addition of cleanup steps. - Use of highly sensitive methods, such as MS.
Protein degradation during sample collection and storage	Proteins in wastewater are unstable and susceptible to enzymatic degradation or chemical modification, especially in warm or variable conditions.	- Immediate cooling and preservation of samples at less than 4 °C. - Rapid processing post-collection to minimize degradation.
Detection of unknown/emerging pathogens	Antigen assays may require a priori knowledge of pathogen proteins and validated antibodies.	- Use of shotgun proteomics for unbiased screening. - Establishment of adaptable platforms using aptamers or nanobodies that can be rapidly customized to new antigens.
Limited reference databases for pathogen proteins in environmental matrices	Annotated databases used for peptide identification are often built from clinical or pure-culture data. In wastewater, sequences may differ (e.g., due to strain variation or environmental degradation), leading to missed identifications.	- Construction of WBE-specific peptide libraries (e.g., environmental metaproteome references). - Integration of metagenomic and proteomic data to build more accurate hybrid search databases.
Reproducibility across sites and batches	Variation in sample collection, processing, digestion efficiency, and instrument conditions may bring with it inconsistencies in inter-lab or time-series data.	- Use of spiked-in quality control peptides. - Implementation of system suitability tests before and during analysis. - Development of standard reporting guidelines for environmental proteomics.
Data analysis and interpretation bottlenecks	Large proteomic datasets require complex processing, statistical normalization, and biological interpretation—especially challenging in heterogeneous wastewater samples.	- Use of automated pipelines (e.g., Skyline, DIA-NN, MaxQuant). - Application of machine learning for pattern recognition in immune signatures or outbreak trends. - Combination of proteomic signals with metadata (e.g., flow, population, climate) to contextualize results.
Limited multiplexing in non-MS platforms (e.g., biosensors)	Many immunosensor devices are still single analyte, limiting their utility for simultaneously monitoring multiple pathogens or immune markers.	- Development of chip-based or microfluidic biosensor arrays for multiplex detection. - Incorporation of aptamer/nanobody strategies for broader target coverage with minimal cross-reactivity.
Regulatory and translational gaps	Lack of regulatory frameworks or clinical validation for proteomic WBE tools hampers adoption by public health agencies.	- Promotion of cross-disciplinary partnerships (academia, public health, regulatory bodies). - Conduction of pilot programs with clear endpoints for validation and integration into surveillance infrastructure.

Abbreviations: MS, mass spectrometry; WBE, wastewater-based epidemiology.

disease surveillance. LC-MS/MS has facilitated the identification of several candidate biomarkers for wastewater-based monitoring. For example, vitamin D-binding protein and monocyte chemoattractant protein are both overexpressed in renal disease, while gelsolin—cleaved after cell injury—produces t-gelsolin, a negative acute-phase protein [23]. Additional urinary biomarkers include prostate-specific antigen, CRP, interleukin (IL)-6, IL-8, podocin, anterior gradient protein 2, and uromodulin, all elevated in various pathological conditions [15]. High-molecular-weight proteins, such as α-amylase and calprotectin, indicate stress and inflammation [9], while cardiac troponin I, cystatin C, α-fetoprotein, and normetanephrine have been recommended for cardiovascular and cancer surveillance [24].

While MS-based methods offer detailed detection of disease-related proteins and pathogen peptides, faster, portable tools are needed for near-real-time WBE. Biosensors meet this need by enabling rapid on-site detection using biological recognition elements, such as antibodies, aptamers, or enzymes, attached to a transducing surface [25]. Upon binding the target, these devices generate electrical or optical signals, and both electrochemical and optical biosensors have demonstrated sensitivity down to the femtogram-per-milliliter level, even in diluted wastewater. Electrochemical immunosensors, in particular, exhibit high specificity in complex matrices when antifouling strategies are employed [26].

Immunosensors, which rely on antibody–antigen interactions, are increasingly used to detect pathogens in wastewater. By substituting different antibodies, the same platform can be adapted to various pathogens, making it useful for both clinical diagnostics and environmental surveillance. However, validation with real environmental samples is still emerging. Early field demonstrations, such as microfluidic paper devices for norovirus detection [27] and paper-based biosensors for SARS-CoV-2 in wastewater [28], highlight both potential and challenges, particularly the need for extensive sample cleanup to remove humic acids and solids that can distort results [28,29]. Several recent examples illustrate biosensor progress. An automated chemiluminescence enzyme immunoassay adapted for use in market wastewater demonstrated high sensitivity (100%) and specificity (66.7%) compared to reverse transcription quantitative PCR (RT-qPCR). It achieved a nanogram-per-liter detection limit (LoD) and processed up to 120 samples in just 35 min. Notably, the assay reported no false negatives, although some false positives were observed [13]. A portable electrochemiluminescent immunosensor quantified the SARS-CoV-2 spike S1 protein, with high recovery and reproducibility in both river and urban wastewater [30]. A nanobody-based immunosensor detected the rotavirus VP6 antigen with an LoD of 0.02 ng L^{-1} in 25 min [31]. Biosensor

applications also extend to pharmaceutical monitoring. For example, an ultrasensitive optical fiber immunosensor was used to measure the antibiotic ciprofloxacin in raw sewage, achieving high recovery and precision after basic filtration and incubation [32]. Collectively, these studies demonstrate that biosensors can provide semiquantitative results in reduced time. Their speed and portability make them ideal for daily screening at WWTP inlets or during large public events. However, challenges remain, including cross-reactivity, matrix complexity, and the limited single-analyte design of most devices. Post-fabrication selectivity testing and robust cleanup protocols are essential to avoid false positives and signal interference.

Alongside biosensors, immunoaffinity-based techniques have emerged to enrich low-abundance proteins in wastewater [4]. These methods use specific antibodies to isolate target proteins, thereby improving the signal-to-noise ratio, while the enriched proteins can then be analyzed using MS. Immunoprecipitation, for example, uses antibody-coated beads to extract proteins from raw or processed wastewater samples [7].

LC-MS/MS remains a standard for high-resolution, ultrasensitive analysis, routinely enabling the quantification of both proteins and small molecules in wastewater at subpicogram levels. In one proof-of-concept, ultra-high-performance LC (UHPLC), coupled with a triple-quadrupole MS, monitored SARS-CoV-2 nucleocapsid protein and CRP, achieving nanogram-per-liter detection limits, 80–100% accuracy, and 2.8% precision [17]. LC-MS/MS also supports pharmaceutical surveillance. Following solid-phase extraction, a 12-min LC-MS/MS run quantified multiple drugs in WWTP effluent with LoD as low as 0.002 ng L^{-1} and high measurement precision, with variability remaining below 5% for most analytes [33]. Simpler protocols, such as vortex-and-filter direct injection, have also shown acceptable sensitivity and reproducibility for antimicrobial detection [34].

When coupled with immunocapture, LC-MS/MS offers exceptional sensitivity, specificity, and reproducibility, making it suitable for confirmatory testing, trace-level quantification, and longitudinal monitoring. It also enables consistent quantification of low-abundance peptides across labs [35] and can distinguish protein isoforms in complex matrices, such as plasma [36] and river water [37]. However, these advantages come at the cost of expensive instrumentation, skilled personnel, high solvent consumption, and time-intensive workflows.

These techniques are not competitors but collaborators. In a practical WBE pipeline, cost-effective biosensors may be used to screen hundreds of samples weekly, identifying targets for confirmation via immunoaffinity LC-MS/MS. In parallel, LC-MS/MS analyses can be employed periodically to expand biomarker discoveries and refine surveillance panels. Table 3 summarizes the key trade-offs that influence strategy deployment. These comparisons support the idea that hybrid workflows may become the gold standard for trace-level, population-wide immunoproteomic surveillance.

Overall, these advances underscore the importance of integrating proteomics with immunological and biosensing techniques, such as immunoassays, immunosensors, chemiluminescence assays, and immunoaffinity enrichment, for comprehensive wastewater monitoring (Fig. 1).

5. Clinical outcomes linked to wastewater proteins

Environmental proteomics has advanced rapidly in recent years, emphasizing the importance of incorporating an immunological perspective to support environmental monitoring, public health, and clinical diagnostics. Table 4 summarizes the evolution of wastewater proteomics, from early feasibility studies using polymer probes to advanced techniques, such as targeted proteomics, LC-MS/MS (DIA mode), and biosensors, and links each method to key public health findings.

Recent advances in wastewater proteomics have identified an expanding list of disease-related proteins, such as S100A8, defensins, and uromodulin [7], which offer valuable insights into population health. According to UniProt (https://www.uniprot.org/), S100A8 is a calcium- and zinc-binding protein that regulates inflammation and immune responses. It is elevated at inflammation sites and in patients with conditions such as rheumatoid arthritis, cystic fibrosis, inflammatory bowel disease, and various cancers (e.g., gastric, esophageal, colon, and pancreatic). Its presence in wastewater may reflect widespread inflammation linked to infection or chronic diseases. The detection of defensins, which are antimicrobial peptides critical for innate immunity, suggests active infection in the population. Uromodulin, produced by kidney tubule cells, is a marker of renal function, and elevated levels in wastewater may indicate population-wide renal stress or early pathology. Noninvasive monitoring of these biomarkers through wastewater can reveal trends in subclinical disease and support more effective resource allocation by clinicians and policymakers.

Beyond inflammation and renal markers, WBE is also used to detect indicators of chronic noncommunicable diseases. A recent scoping review identified five urinary proteins (cardiac troponin I, cystatin C, α-fetoprotein, normetanephrine, and MAST4) as potential WBE biomarkers for cardiovascular disease and cancer [38]. Prevalence-adjusted models have shown that their concentrations in community wastewater can be detected via LC-MS/MS, supporting the passive monitoring of subclinical heart disease and cancer risk.

Recent research has also highlighted the value of monitoring immunoglobulins in wastewater [9–11]. Antibodies are essential for immune protection, and measuring them can provide insight into community-level immune status. IgG, associated with long-term immunity, may indicate prior infection or vaccine-induced protection. In contrast, IgA, which predominates at mucosal surfaces, often reflects recent or ongoing infections, particularly in the respiratory or gastrointestinal tract. Monitoring antibody titer changes over time could help estimate infection rates and vaccination coverage. Correlating these profiles with immunization campaigns (e.g., influenza and COVID-19) could also identify immunity gaps in near real time.

The integration of proteomic and immunological data offers transformative advances in WBE [6]. Traditionally, wastewater monitoring has focused on detecting viral genetic material. The addition of immunological markers provides a multidimensional view. For instance, the detection of influenza-specific antibodies can signal impending outbreaks before clinical cases arise. Simultaneously, MS analysis of viral proteins can confirm active infections. The SARS-CoV-2 pandemic demonstrated how quickly WBE can adapt to new pathogens. As new immunoassays become available, they can be incorporated into surveillance pipelines for early detection.

A multipathogen profiling strategy combining proteomic (e.g., S100A8, defensins), immunological (e.g., IgG, IgA), and molecular (e.g., qPCR, metagenomics) markers offers a more comprehensive picture of community health. This approach is particularly beneficial in resource-limited settings, where multiple pathogen-specific assays may not be feasible.

Another promising advance in WBE is the integration of wastewater findings with clinical and hospital data to refine epidemiological models [15]. For example, rising levels of S100A8 or defensins in wastewater could prompt a review of local hospital admissions for inflammation-related illnesses. Similarly, elevated

Table 3
Comparative performance and operational trade-offs between immunosensors and LC-MS/MS in wastewater-based epidemiology.

Technology	Sensitivity/Specificity	Throughput/Speed	Key Trade-offs
Immunosensors	Typical LoD: ~0.02–1.00 ng L^{-1}, depending on antigen; specificity depends on antibody.	Preprocessing time depending on preconcentration or cleanup steps (min to hours) plus 15–35 min per run; portable/on-site capability.	Rapid, low-cost, and field-compatible, but vulnerable to cross-reactivity, false-positives, and matrix interferences, and often restricted to single-analyte formats.
LC-MS/MS	LoDs from low-pg L^{-1} to μg L^{-1} for small molecules and proteins; high specificity; able to resolve isoforms.	Preprocessing time depending on digestion, chromatography and enrichment steps (hours) plus 10–60+ min per run; centralized lab required.	Extremely precise and multiplexed, but slower, costly, and requires skilled personnel.

Abbreviations: LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LoD, limit of detection.

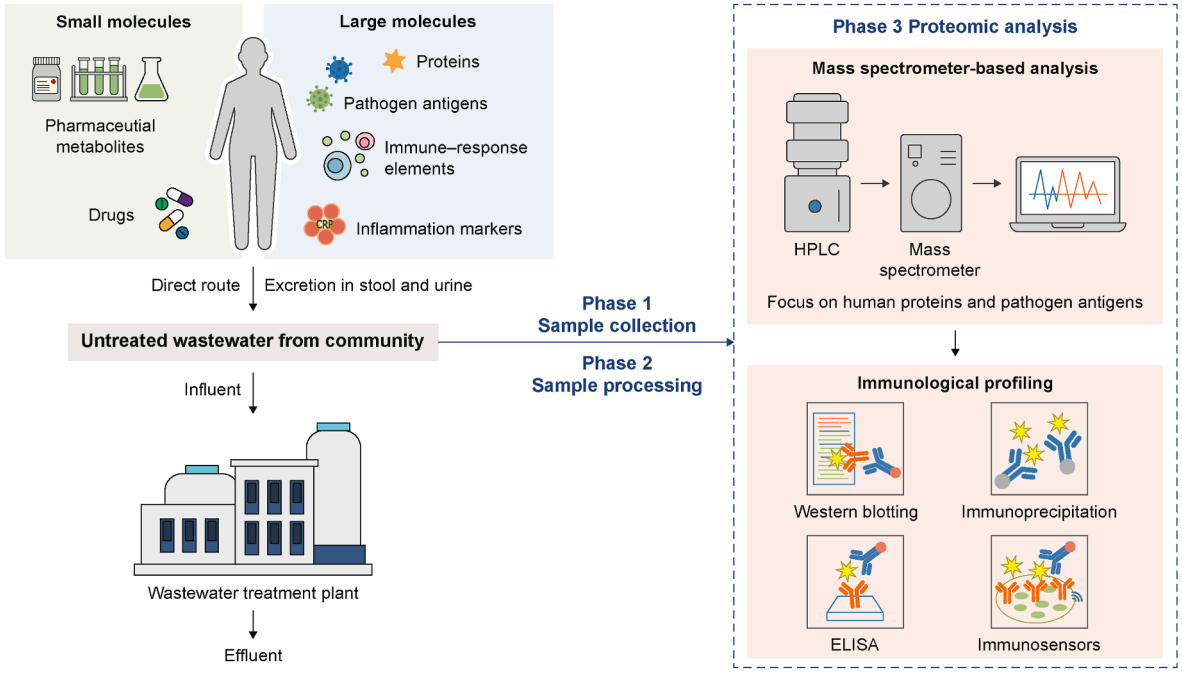

Fig. 1. Integrated pipeline for wastewater-based immunoproteomic surveillance of community health. The left side of the figure illustrates how diverse waste products from a community—including small molecules (e.g., pharmaceuticals and illicit drugs) and large biomolecules (e.g., broader protein biomarkers, such as pathogen antigens; inflammatory markers, such as C-reactive protein (CRP); and immune-response elements, such as immunoglobulins and cytokines)—enter the wastewater system either directly or via excretion in stool and urine. The diagram illustrates the process of collecting samples from the influent of a wastewater treatment plant, followed by sample preparation for protein analysis. Proteins are digested into peptides and analyzed via high-performance liquid chromatography (HPLC) coupled with mass spectrometry, with the resulting spectra matched to protein databases. Parallel immunological approaches complement proteomics by verifying antibody specificity, detecting pathogens, and enriching low-abundance targets. Together, these methods provide a multidimensional, non-invasive view of community health, capturing the presence of pathogens as well as immune responses within an integrated wastewater-based monitoring framework. ELISA: enzyme-linked immunosorbent assay.

pathogen-specific antibodies could be linked to recent vaccination drives or outbreak response efforts. Such correlations improve model accuracy and support earlier, more targeted public health interventions.

Hospital sewage, in particular, is a rich source of pathogens, including bacteria (e.g., *Escherichia coli*, *Salmonella*, *Proteus*, *Shigella*, and *Staphylococcus aureus*), viruses (e.g., norovirus, adenovirus, and rotavirus), and parasites (e.g., *Microsporidium*, *Cryptosporidium*, and helminths) [39]. Effective monitoring and mitigation of these pathogens are essential for protecting both public and environmental health.

Immunosensors have emerged as effective tools for rapid on-site pathogen detection. By utilizing specific recognition elements, immunosensors can rapidly identify microbial targets, thereby complementing existing WBE strategies. For example, they can serve as early-warning screens, followed by confirmatory proteomic or molecular assays. This layered diagnostic workflow enables rapid yes/no decisions, prioritizes sample processing, and reduces overall turnaround time. Only positive samples require follow-up analysis, thereby optimizing both resources and speed. Thus, immunosensors not only enhance our understanding of disease dynamics in healthcare environments but also support effective wastewater management.

6. From genetic signatures to infection markers in wastewater monitoring

Nucleic acid-based methods, especially RT-qPCR and digital PCR, remain central to viral monitoring in wastewater. These techniques offer excellent analytical sensitivity (~1–10 genome copies mL^{-1}), high specificity, and the ability to multiplex (2–4 targets), delivering results in under 6 h. However, they also face practical limitations [29,40]. High equipment and reagent costs limit their use in low-resource settings, and environmental inhibitors in wastewater can compromise both sensitivity and reliability.

Isothermal methods, such as nucleic acid sequence-based amplification, reduce assay time (15–60 min) and simplify equipment requirements. However, they remain vulnerable to matrix inhibition and often lack robustness for reliable field deployment.

Table 4
Overview of analytical workflows and outcomes in wastewater proteomic research.

Study/approach	Matrix/source	Analytical methods	Key findings	References
Analysis of large-volume samples using a polymer probe-based approach	Influent/effluent WWTP samples	Untargeted proteomics LC-MS/MS	Overcame high-volume processing challenges; demonstrated feasibility of analyzing proteins from diverse sources (bacteria, plants, and animals, including humans).	[8], [9]
High-throughput workflows for fraction-specific analysis	Soluble and particulate phases of wastewater	Untargeted proteomics LC-MS/MS	Showed distinct protein profiles in soluble (Chordata-enriched) and particulate fractions (bacteria-enriched). Identified two main protein sources (human excreta and livestock byproducts) as well as bacterial, plant, and human proteins.	[7]
Hybrid small- and large-molecule screening	WWTP influent	LC-MS/MS	Offered a multidimensional perspective on community health by simultaneously detecting metabolites (e.g., drugs) and proteins (e.g., inflammation markers).	[14], [15]
Searching for biomarkers in wastewater	Influent WWTP samples	Untargeted proteomics LC-MS/MS (DIA)	Detected 44 human proteins associated with infectious diseases (immunoglobulins, cytokines, chemokines). Correlated these protein levels with local COVID-19 incidence.	[11]
Detection of disease-related proteins in wastewater	Influent WWTP samples	Untargeted proteomics LC-MS/MS	Identified unique peptides from eight SARS-CoV-2-related proteins prior to clinical case reporting. Highlighted pp1ab as an alternative biomarker for COVID-19 surveillance.	[12]
Proof-of-concept study on the detection of pathogen antigens and host-derived proteins	Influent WWTP samples (days with varying SARS-CoV-2 prevalence)	Targeted proteomics LC-MS/MS	Concurrently detected viral components and acute-phase biomarkers to support early-warning systems and immunosurveillance. Demonstrated the utility of targeted MS beyond traditional PCR-based methods.	[17]
Biosensor for the detection of specific antigens	Wastewater samples from fresh markets	Automated CLEIA	Achieved 100% sensitivity and 66.7% specificity for SARS-CoV-2 antigen compared to RT-qPCR, thereby confirming the feasibility of automated CLEIA in wastewater monitoring.	[13]
Biosensor for the detection of specific antigens	River and urban wastewater	Carbon nanodot-amplified ECL immunosensor	Demonstrated high specificity and stability in detecting SARS-CoV-2 spike S1 protein. Provided an effective early-warning tool for viral circulation in the population.	[30]
Integrating MS proteomics with immunological assays	Samples from central WWTP and building-scale sites; solid and soluble fractions	ELISA, Western blot, LC-MS/MS	Confirmed the presence of intact, functional antibodies (IgG, IgA) in both neighborhood-scale and centralized wastewater. Demonstrated avidity for SARS-CoV-2 and influenza A.	[10]

Abbreviations: CLEIA, chemiluminescence enzyme immunoassay; DIA, data-independent acquisition; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; LC-MS/MS, liquid chromatography with tandem mass spectrometry; WWTP, wastewater treatment plant.

Meanwhile, high-throughput sequencing (HTS) enables broad, unbiased detection (including novel viruses) but is hindered by high costs, long turnaround times, complex bioinformatics, and susceptibility to elevated error rates (especially with long-read platforms).

Crucially, none of these molecular techniques assesses viral infectivity; that is, they detect genetic material, not viable infectious particles. This distinction has major public health implications. A meta-analysis of SARS-CoV-2 in wastewater revealed that while viral ribonucleic acid (RNA) may persist for days, intact infectious virions degrade 3–10 times faster [41]. As a result, RNA-based methods may detect noninfectious remnants or fail to detect viable viruses when RNA has degraded.

Protein-centered assays offer a complementary perspective by targeting viral antigens (e.g., capsid or spike proteins) and host-response proteins. These markers provide more biologically relevant insights into active infections and immune responses. Additionally, standard proteomic cleanup steps remove many of the inhibitors that affect PCR reliability.

For example, an LC-MS/MS study across 10 Spanish WWTPs demonstrated a clear quantitative link between wastewater protein markers and clinical COVID-19 trends. Researchers identified 44 infectious disease-related proteins that showed a significant correlation with case numbers reported two weeks later ($\rho = 0.56$, $p = 0.04$), with immunoglobulins exhibiting an even stronger correlation ($\rho = 0.64$, $p = 0.02$) [11]. While these associations weakened after mass vaccination, protein levels still peaked approximately two weeks before cases were reported, and IgA levels rose later in the pandemic, aligning with increased vaccination coverage. In a separate study, LC-MS/MS was used to monitor both SARS-CoV-2 nucleocapsid protein and the inflammatory marker CRP in wastewater [17]. Population-normalized daily loads of SARS-CoV-2 rose from 2.6 to 4.7 mg per day per 1000 inhabitants between low- and high-prevalence days, closely tracking infection rates. Although CRP levels remained lower than expected, possibly due to biological or methodological factors, the dynamics of SARS-CoV-2 proteins reinforced the utility of proteomics for tracking real-time public health trends.

Aptamer-based biosensors also show strong potential. A systematic review found that they can detect SARS-CoV-2, influenza, and norovirus at concentrations ranging from femtomolar to picomolar using submilliliter sample volumes. They deliver results in 30 min or less, with dynamic ranges spanning four to five orders of magnitude [42].

Recent advances have demonstrated that a dual approach (MS + genomics) can identify both known and previously unknown public health threats. For example, a study using LC-MS/MS to track symptom-related pharmaceuticals and RT-qPCR for respiratory viruses across 64 WWTPs found that pharmaceutical levels generally mirrored viral loads [43]. However, unexplained drug surges, later linked to rhinovirus and pertussis outbreaks, were missed by PCR alone. Similarly, a pilot study monitored frequently prescribed antibiotics and antibiotic resistance genes (ARGs) in wastewater over 12 months [44]. LC-MS/MS revealed actual drug-use patterns that diverged from prescription data, while qPCR showed that total antibiotic load correlated with at least one ARG, indicating selection pressure. The study also captured a post-COVID-19 decline in macrolide and quinolone use, demonstrating WBE's sensitivity to shifts in prescribing behavior and its potential for early surveillance of antimicrobial resistance (AMR). In parallel, a high-throughput, direct-injection LC-MS/MS method has been validated for quantifying hundreds of antimicrobials and their metabolites in wastewater, making it ideal for integration into proteomic or genomic pipelines for

Table 5
Comparison of proteomic and nucleic acid-based workflows for wastewater-based epidemiology.

Aspect	Nucleic-acid-based methods	Proteomic methods
Primary targets	RNA/DNA	Antigens, host proteins (e.g., CRP, IgG), small molecules
Sensitivity	High (1–10 genome copies mL^{-1})	High (femtomolar–picomolar)
Time to result	Minutes for NASBA to hours for PCR	Minutes for biosensors to hours for LC-MS/MS
Multiplexing capability	2–4 targets per run for PCR to hundreds for HTS	Moderate for biosensors to hundreds–thousands for LC-MS/MS Relevantly, LC-MS/MS allows parallel analysis of both proteins and small molecules
Infectivity insight	No (does not indicate viability)	Yes (antigens reflect intact, potentially infectious particles)
Resistance to inhibitors	Susceptible to inhibitors in wastewater	Better resistance due to proteomic clean-up steps
Cost and accessibility	High costs, lab infrastructure needed	Moderate to high (biosensor lower cost than LC-MS/MS)

Abbreviations: CRP, C-reactive protein; DNA, deoxyribonucleic acid; HTS, high-throughput sequencing; IgG, immunoglobulin G; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; NASBA, nucleic acid sequence-based amplification; PCR, polymerase chain reaction; RNA, ribonucleic acid.

chemical–AMR surveillance [34]. A related UHPLC-MS/MS study found significantly higher antibiotic loads in hospital wastewater compared to urban WWTP influent, reinforcing hospitals as key contributors to AMR and ecotoxic risk [45].

At the same time, nucleic acid-based methods continue to expand WBE's reach across a wider range of pathogens. RT-qPCR has been used to detect monkeypox virus (MPXV) deoxyribonucleic acid in both influent and sludge samples from WWTPs [46]. Separately, viral probe-based sequencing has identified over 450 pathogenic viruses, including many that were previously undetected, in longitudinal wastewater samples [47]. These sequencing reads correlated well with clinical data for SARS-CoV-2, influenza, and MPXV, confirming their epidemiological relevance [47].

Seasonal influenza strains, such as H3N2 and H1N1, have been tracked via RT-qPCR and HTS [48], with wastewater viral loads generally reflecting clinical case trends [49]. However, the detection of H5N1 RNA in wastewater without corresponding hospitalizations illustrates the limitations of genome-only approaches for determining infectious risk [49]. In this context, recent innovations in microfluidic electrochemical immunosensors, capable of multiplex antigen detection for high-risk influenza A subtypes (H1N1, H5N1, H7N9) [50], underscore the importance of proteomics as a strong complement to genomics in WBE.

Taken together, these advances reflect a growing consensus: while genomic tools offer broad and sensitive detection, proteomic methods add biologically meaningful, infection-level resolution. With antigen detection for SARS-CoV-2 now feasible via LC-MS/MS and biosensors, similar workflows may soon be adapted for emerging targets, such as MPXV and novel influenza variants.

As these detection platforms mature, they promise to bridge the gap between pathogen presence and actual infectious risk, bringing WBE closer to actionable, real-time public health interventions. Table 5 provides a comparative overview of proteomic and nucleic acid-based workflows, outlining their respective strengths and limitations.

7. Conclusions

The integration of proteomic and immunological methods into WBE provides a powerful, noninvasive platform for population-level health surveillance. It supports the detection of emerging infectious threats, the monitoring of subclinical disease trends, and the assessment of community immune responses, including vaccination coverage and efficacy.

As high-throughput MS, multiplex immunoassays, and biosensor technologies continue to advance, the precision, speed, and scope of WBE will expand. Near-real-time pathogen detection, combined with the quantification of disease-relevant proteins and immunoglobulins, offers unprecedented potential to anticipate outbreaks and chronic disease burdens before clinical symptoms emerge. Aligning wastewater data with hospital records and public health databases can refine epidemiological models, enhancing both the timeliness and accuracy of interventions.

Certainly, technical challenges remain before the full potential of immunoproteomics can be realized in routine WBE applications. For example, LoDs still constrain the identification of low-abundance proteins, especially those showing subtle changes in dilute wastewater. Chromatographic limitations, particularly in distinguishing immunoglobulin subclasses or heavily glycosylated proteins, can introduce quantification uncertainties. The complex composition of wastewater, laden with organic and inorganic interferents, necessitates rigorous sample preparation and advanced fractionation protocols.

Addressing these obstacles will require continuous adjustments in sensitivity and specificity. Workflow optimization for protein and antibody enrichment is also necessary, along with more robust bioinformatics tools to manage large and complex datasets. Continued collaboration among analytical chemists, bioinformatics experts, biomedical researchers, and public health practitioners will be essential for translating these innovations into high-impact, operational WBE systems.

Looking forward, the next frontier will involve expanding multi-omics integration in WBE. This will include not only proteomics and immunology but also metagenomics, metabolomics, and beyond. This comprehensive framework could enhance understanding of disease transmission, immunity, and population health. In turn, such insights may inform more targeted public health strategies and more efficient resource allocation. Ultimately, by linking environmental surveillance with clinical medicine, WBE stands poised to become a proactive, cost-effective, and community-centered pillar of global disease monitoring. As this integration advances, establishing clear ethical guidelines and governance frameworks will be essential, especially when immunological markers are analyzed in decentralized or building-level wastewater systems.

CRediT authorship contribution statement

Jaxaira Maggi: Writing - Original Draft, Visualization, Investigation, Conceptualization. **Joaquin Abian:** Writing - Review & Editing, Validation. **Antoni Ginebreda:** Writing - Review & Editing, Validation. **Damià Barceló:** Writing - Review & Editing, Validation, Supervision. **Montserrat Carrascal:** Writing - Review & Editing, Visualization, Validation, Supervision, Project Administration, Funding Acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Spanish Ministry of Science and Innovation (MICINN, Spain) (Project nos. PID2020-114065RB-C22 and PID2020-114065RB-C21).

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