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Review Article

Wastewater-Based Surveillance of Antimicrobial Resistance: Current Evidence and Methodological Advances

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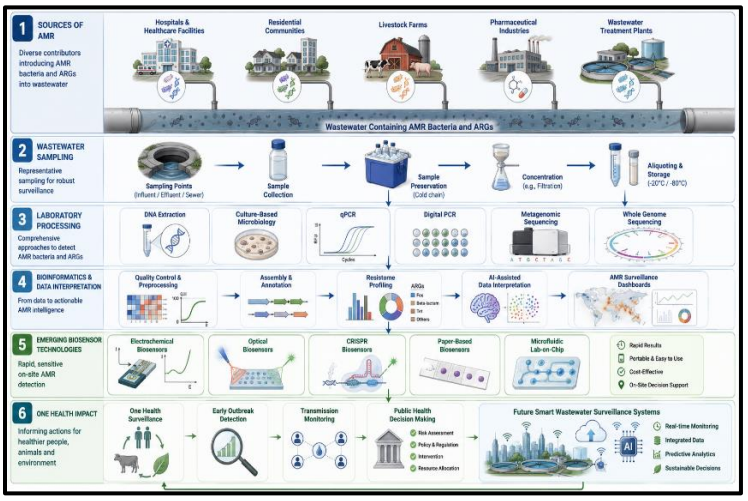
ABSTRACT

Surveillance of antimicrobial resistance (AMR) using the technique of wastewater-based surveillance (WBS) is the most reliable strategy in the One Health paradigm in recent times. Based on wastewater analysis, WBS offers the possibility of the identification of resistance genes in the human community, environment, and animals. This manuscript discusses some recent developments in WBS technologies such as metagenomics, high throughput quantitative PCR (qPCR), and culture methods but brings focus to a significant drawback that exists in all these strategies on-site analysis is missing. In this paper, attempt has been made to formulate a composite surveillance system using some of these techniques mentioned above, such as metagenomics, high throughput qPCR, and electrochemical microfluidic biosensors as one comprehensive strategy of AMR surveillance using WBS. In contrast to other WBS strategies based on sequencing in laboratories, the new proposed strategies will seek to conduct on-site analysis, hot-spot identification using artificial intelligence, and environmental surveillance using lab on a chip electrochemical biosensor techniques.

Keywords — Wastewater-based surveillance (WBS); Antimicrobial resistance (AMR); Biosensors; Electrochemical biosensor; Lab-on-a-chip; Microfluidics; Antibiotic resistance genes (ARGs); One Health; Real-time detection; Artificial intelligence (AI); Wastewater-based epidemiology (WBE); Resistome; Environmental monitoring; Predictive surveillance



GRAPHICAL ABSTRACT

I. INTRODUCTION

The effective monitoring of antibiotic resistance related to hospitals, communities, and even globally can be done today through the use of sewage monitoring. Current tools include Metagenomic analysis, qPCR techniques, cultivation techniques, and analytics which are used for analyzing resistome profile, hotspots determination and contributing towards one health policies development. The presence of antibiotic-resistant bacteria, resistance genes, and all other mobile resistance conferring factors including plasmids, transposons, environmental drug residues is highly concentrated at hospitals and sewage treatment plants. This enables them to persist and perhaps spread to the environment. Monitoring of the resistance to antimicrobials among the

population can also be done without needing to test individuals by analyzing the data from sewage collected from both urban areas and hospitals. (Rodríguez et al., 2021; Majeed et al., 2021; Ramos et al., 2024; Ballén et al., 2025; Silvester et al., 2025).

Antimicrobial resistance can be predicted globally by analyzing sewage which have undergone no treatment as these studies show unique trends in distribution of the resistance genes depending on economic and sanitary conditions of a particular place (Hendriksen, R., et al. 2019).

II. CURRENT WASTEWATER-BASED SURVEILLANCE APPROACHES

Metagenomics provides a way to understand resistomes and mobilomes and their associated hosts. It pays attention to pathogenic organisms such as ESKAPE and new enteropathogens. Furthermore, we gain knowledge about the association of the genes responsible for antibiotic resistance among bacteria such as bla, mcr and tet(X) (Rajput et al., 2025; Knight et al., 2024; Ramos et al., 2024; Rodríguez et al., 2021). A schematic framework detailing the sampling pathways and standard processing tools of WBS is structured in **Figure 1**, with an initial summary of core performance factors outlined in the **Key Methodological Contrasts** table.

The methods of detection of these genes are HT-qPCR and target specific PCR techniques. They are quite sensitive methods but do not always yield reliable results due to issues such as primer mismatches. The combination of qPCR and metagenomics has proved to provide reliable information. It becomes easy to identify high-risk genes including carbapenemases by using culture enrichment (Knight et al., 2024; Elbait et al., 2024; Davis et al., 2023; Majeed et al., 2021). A deeper parameter-by-parameter evaluation evaluating limits, costs, and detection times of these standard techniques compared against innovative approaches is presented in **Table 1**.

Methods like read and metatranscriptomic methods are also used to connect the resistance genes to the hosts and to see how they are expressed. Through this, the risks associated with metagenomics and resistance genes are understood, as well as what is meant for the hosts—such as the ESKAPE(E) pathogens and other bacteria in which these genes (including *bla*, *mcr*, and *tet(X)*) are found (Acosta et al., 2025; Knight et al., 2025; Rodríguez et al., 2021).

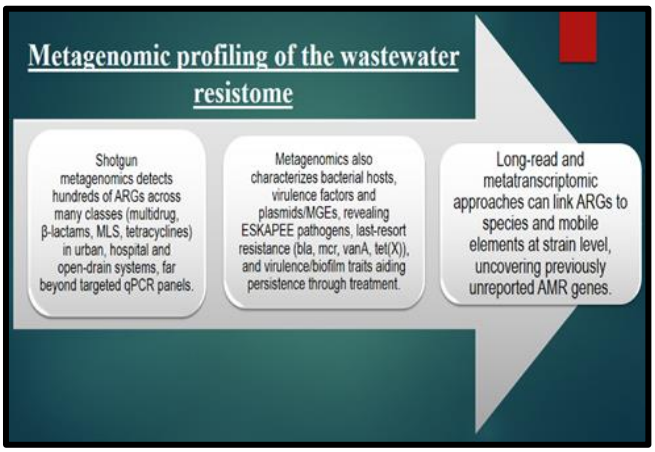


Figure 1. Wastewater-Based Surveillance Framework for AMR Detection. A schematic diagram of WBS depicting the sampling of sewage from hospitals and communities, and further analysis using metagenomics, quantitative PCR, and culture methods. The diagram emphasizes the detection of ARGs, data analysis, and implementation of WBS in One Health surveillance to monitor AMR in the population.

Key methodological contrasts

Method	Strengths	Limitations
HT-qPCR	Highly sensitive for targeted ARG quantification	Detects only predefined ARGs
Short-read metagenomics	Broad resistome profiling with taxonomic context	Limited ARG–host linkage
Long-read metagenomics	Resolves ARG–host–plasmid linkage; detects novel ARGs	Higher cost; lower sequencing depth

Table 1. Comparison of Major Methodological Approaches in WBS for AMR

Method	Sensitivity	Detection Time	Cost	Throughput	Strength	Limitation
Metagenomics	Very high	24–72 h	Very high	High	Comprehensive resistome profiling	Expensive, computationally intensive
HT-qPCR	High	3–6 h	Moderate	Moderate–High	Quantitative ARG detection	Primer bias, limited targets
Culture-based methods	Moderate	24–96 h	Low–Moderate	Low	Viable organism isolation	Underestimates unculturable diversity
Meta-transcriptomics	High	24–48 h	High	Moderate	Functional gene expression analysis	RNA instability
Proposed biosensor platform	Moderate–High*	10–30 min	Potentially low	High	Rapid field-deployable multiplex detection	Requires environmental validation

*Performance estimates for biosensor systems are derived from currently reported electrochemical ARG bio-sensing studies and remain subject to wastewater matrix optimization.

Table 2. Key Antimicrobial Resistance Genes Detected in Wastewater

Gene Category	Example Genes	Antibiotic Class	Clinical Significance
Carbapenem resistance	blaKPC, blaNDM, blaOXA-48	β-lactams	Critical WHO priority
Colistin resistance	mcr-1 to mcr-9	Polymyxins	Last-resort antibiotic resistance
ESBLs	blaCTX-M, blaTEM	Cephalosporins	Common hospital infections
Tetracycline resistance	tet(X)	Tetracyclines	Widely distributed
Vancomycin resistance	vanA, vanL	Glycopeptides	Enterococcal infections

III. PROPOSED INTEGRATED SURVEILLANCE FRAMEWORK

Although there have been attempts to apply metagenomics, HT-qPCR, and bio-sensing techniques separately to AMR surveillance studies, a synergistic application of all three approaches in an integrated wastewater-based surveillance system architecture needs further development. Current WBS studies rely heavily on centralized laboratory processing, including sequencing and later analysis of data. The proposed framework expands upon current technologies and suggests a novel layered approach to AMR surveillance, combining:

- A high-resolution resistome analysis based on metagenomics,
- Validation of results via HT-qPCR technology,
- ARG detection at the field level via electrochemical biosensors,
- AI-based analysis and hotspot/outbreak prediction.

The new framework incorporates an innovative translational approach where Lab-On-A-Chip bio-sensing systems can serve as decentralized screening tools allowing for immediate acquisition of surveillance data directly from the wastewater matrix. This approach would facilitate rapid detection of ARGs including blaNDM, blaKPC, blaOXA-48-like, and mcr variants. The clinical priority classification and genetic profiles of these target biomarkers are summarized comprehensively in **Table 2**.

As opposed to current surveillance approaches which focus predominantly on retrospective actions, the framework highlights an approach based on predictive/operational surveillance through the incorporation of bio-sensing, environmental epidemiology, and machine-learning-based analytics.

IV. AI-DRIVEN RESISTOME ANALYTICS

Research employs network analysis & statistical tools for correlations between genera, antibiotics resistance genes, mobile genetic elements and environmental factors in order to pinpoint indicator taxa and resistome risk scores for hospitals and community settings. National & global research uses machine learning techniques to correlate AMR profiles with socioeconomic, environmental and antimicrobial consumption variables that would be used in estimating the bacterial gene load in various countries (Malcom & Bowes, 2025; Rajput et al., 2025; Knight et al., 2024; Ramos et al., 2024; Davis et al., 2023; Majeed et al., 2021; Rodríguez et al., 2021; Hendriksen et al., 2019).

When studying WWTP samples, the genera *Aliarcobacter*, *Acinetobacter*, *Acidovorax*, and *Aeromonas* stood out due to their abundant and diverse content of antimicrobial resistance genes

(ARGs). Aliarcobacter possessed a high level of resistance to Sulphonamides and Polymyxins. *Acinetobacter* and *Aeromonas* were the richest in their abundance of resistance to beta-lactams. Antibiotic resistance genes were found in ESKAPE(E) pathogens – *Acinetobacter* possessed several occurrences of blaOXA and ampC, *Pseudomonas* included blaOXA, blaBEL, blaPDC, and ampC, while *Enterobacteriaceae* contained blaCMY, blaACT, blaGOB, and ampC and Enterococcus included vanL and vanA (Ramos, B., et al.2024). The primary bacterial genera discovered in these environments and their accompanying resistance types are organized in **Table 3**, while the structural workflow for analyzing this cross-sectional data through neural architectures is detailed in **Figure 2**.

A metagenomics analysis of sewage from Pune, India, sampled between December 2022 and December 2023, investigated the occurrence of antimicrobial resistance (AMR). The study discovered significant variation in AMR throughout the year. Using metagenomic sequencing techniques, 157 different bacterial phyla and 3291 species were detected, among which Proteobacteria was the dominating phylum. There was an evident seasonality in ARGs, with an increased number detected in winter. The study detected 637 different ARGs associated with 29 classes of antibiotics, with multidrug resistance and beta-lactam resistance genes in Enterobacteriaceae dominating among these. AMR gene concentrations varied through the year but increased in the winter, probably because more antibiotics are used to treat respiratory diseases at that time (Rajput, V., *et al.* 2025).

Key Findings from Longitudinal and Geographical Studies

The longitudinal study of WWTPs and sewerages provides an idea about the distribution of antibiotic resistance in a number of environments (Acosta et al., 2025; Ballén et al., 2025; Rajput et al., 2025; Silvester et al., 2025; Velazquez-Meza et al., 2025; Knight et al., 2024; Ramos et al., 2024; Majeed et al., 2021; Rodríguez et al., 2021).

- Hospital-based systems have a different resistance pattern than the community-based systems.
- Open drain systems and regional network have different resistance patterns.

The distribution of resistance occurs at different times and places. Different types of resistance occur in different proportions.

Types of resistances are:

- Multidrug resistance
- Macrolide-lincosamide-streptogramin resistance
- Beta-lactam resistance
- Tetracycline resistance

The environmental condition affects the seasonal change in the distribution of antibiotic resistance genes (ARGs) since these genes are closely linked to viral outbreaks and antibiotic prescription pattern. The diversity and prevalence of resistance in ARG is higher in hospital effluent than the community waste water; besides, it plays the most crucial part in partial removal and resistance overlap (Velazquez-Meza et al., 2025; Knight et al., 2024; Majeed et al., 2021; Rodríguez et al., 2021). The broader environmental interactions and seasonal changes driving these genetic fluctuations are tabulated in **Table 4**.

Figure 2. AI-Driven Wastewater Resistome Analytics Pipeline. A workflow that describes the use of biosensor/metagenomics data along with AI and network analysis techniques to develop AMR hotspot maps, determine transmission routes, and forecast potential outbreaks in hospitals and communities.

Table 3. Dominant Bacterial Genera and Associated ARG Profiles in Wastewater

Genus	Associated ARGs	Resistance Type
Acinetobacter	blaOXA, ampC	β-lactam resistance
Aeromonas	β-lactam genes	Multidrug resistance
Aliarcobacter	Sulphonamide, polymyxin genes	High ARG diversity
Pseudomonas	blaOXA, blaPDC, blaBEL	Broad-spectrum resistance
Enterobacteriaceae	blaCMY, blaACT	Multidrug resistance
Enterococcus	vanA, vanL	Glycopeptide resistance

Table 4. Seasonal and Environmental Trends in AMR (Based on Longitudinal Studies)

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Parameter	Observation
Seasonal variation	Higher ARG abundance in winter
Dominant phylum	Proteobacteria
ARG diversity	>600 ARGs across 29 antibiotic classes
Influencing factors	Antibiotic usage, infections, sanitation
Hotspot sources	Hospital wastewater > community wastewater

V. BIOSENSOR-BASED RAPID DETECTION

In view of significant roles that microbial biofilms play in the infection development and antibiotic resistance, new methods have to be developed for their evaluation in various ecological niches. Combining electrochemical biosensors with microfluidic approaches will lead to the improvement in accuracy, sensitivity, and real-time monitoring. Currently, studies mostly deal with detecting microorganisms and ARGs in clinical and bacterial samples with the help of biosensors; the environmental applications are focused on antibiotics. There is insufficient data regarding the comparison between biosensors and conventional molecular approaches, indicating the need for high-quality research in the area. It is crucial to conduct research and promote biosensors as practical tools for solving public health issues like AMR using the One-Health paradigm. Biosensors have a great potential to become a part of the point-of-care testing for AMR through combining bio-recognition elements with modern materials and microbial enzymes. The progress made in the field of microfluidic lab-on-a-chip systems helps increase the speed of analysis and improves results. Such developments include bio-receptors, novel electrode surfaces, as well as multiplexing strategies used alongside microfluidics (**Figure 3**), allowing for substantial optimization over long-standing structural limits (**Table 5**). (Khan et al., 2025; Abouhagger et al., 2024; Lio et al., 2023).

Table 5. Advantages of Biosensor-Based WBS Over Conventional Methods

Parameter	Conventional Methods	Biosensor-Based Approach
Detection time	Hours to days	Minutes
Equipment	Advanced lab infrastructure	Portable devices
Multiplexing	Limited	High
Field applicability	Low	High
Cost (long-term)	High	Potentially low
Real-time monitoring	No	Yes

VI. OPERATIONAL WORKFLOW FOR FIELD DEPLOYMENT

Possible routes for the actual application of WBS through biosensors could include multistep sampling and decentralized detection. Samples of wastewater collected from hospitals, municipal sewer systems, and wastewater treatment plants could be processed at the early stages by way of filtration and DNA extraction. The next step would involve using biosensor chips with Lab-on-a-Chip technology to detect particular ARGs rapidly. Information provided by biosensors may be transferred wirelessly into centralized monitoring databases for further processing and interpretation by analytical AI programs in combination with metagenomics and qPCR data for the identification of potential hotspots of ARG spread and its temporal dynamics. Depending on the purpose of surveillance, sample collection may take place once per day (in case of hospital monitoring) or weekly (at least). Calibration with the use of standardized controls of ARGs and validation against sequencing techniques should be conducted regularly. Moreover, connection of such approaches with the national public health monitoring system will contribute to greater accuracy of the results.

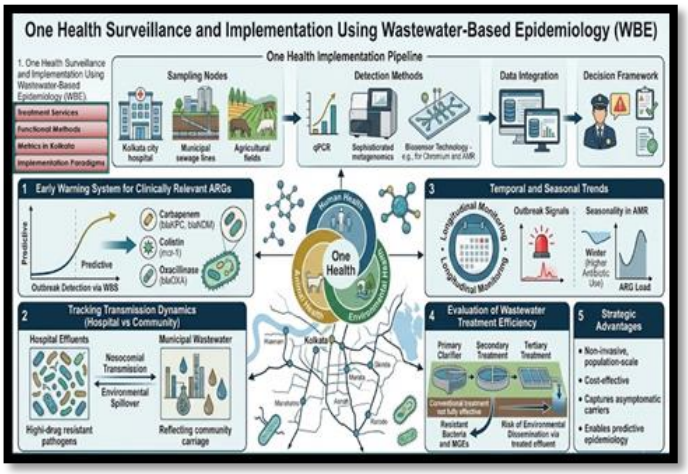

Figure 3. Comparative Workflow of Conventional Vs. Biosensor-Based Detection. A comparative diagram showing conventional AMR detection methods (metagenomics, HT-qPCR, culture-based assays) versus biosensor-based rapid detection. The figure emphasizes differences in time, sensitivity, scalability, and field applicability.

VII. ONE HEALTH SURVEILLANCE

WBS is an emerging method in One Health strategy for surveillance of antimicrobial resistance (AMR) that integrates human, animal, and environmental surveillance. WBS allows detection of microorganisms from various sources, making it easier to detect highly hazardous AMR genes, such as carbapenem or colistin resistance genes. WBS assists in determining the source of AMR spread as well as detecting if there are any possible spill-over events in terms of AMR. Long-term monitoring allows one to determine any temporal changes in AMR spread, detect any outbreak, and measure effectiveness of public health interventions. WBS also allows monitoring of AMR reduction during the process of wastewater treatment as well as persistence of resistant microorganisms in waste water after treatment. For WBS to become a part of One Health AMR surveillance system, it is necessary to conduct multiple sampling, employ various techniques such as quantitative PCR and metagenomics, combine results to inform on antibiotic usage and reduce environmental risks. Major advantages of WBS within One Health framework include cost-effectiveness, population-scale monitoring, and prediction of outbreaks (Balcázar, 2025; Malcom & Bowes, 2025; Rajput et al., 2025; Silvester et al., 2025; Ramos et al., 2024; Davis et al., 2023; Rodríguez et al., 2021).

VIII. CHALLENGES AND FUTURE DIRECTIONS

Implementation Challenges and Frameworks

Review papers point out the need for the use of compatible sampling techniques, composite sampling, standardization of DNA isolation, sequencing depth, and controls. Wastewater-based AMR surveillance is most useful in settings where reporting from the clinical front is poor; however, such a system requires regular expenditure, ethical considerations, and integration within the framework of One Health-based decisions (Balcázar, 2025; Kumar et al., 2025; Malcom & Bowes, 2025; Davis et al., 2023; Rodríguez et al., 2021).

Environmental variability is still the major challenge in achieving accurate WBS AMR surveillance. Variation in antibiotic usage due to seasonality, dilution due to rainwater, composition of the waste itself, industrial discharges, and microbial communities will definitely affect the abundance of the ARGs. Also, there is variation across the geography with regard to sewer network construction, sampling strategy, and even the water treatment plant used. Lack of universal guidelines on sampling, DNA extraction, normalization, sequencing depth, and quantification of ARGs will hinder data standardization in global WBS studies. A breakdown of these categorical challenges alongside concrete technological and methodological directions to mitigate them is detailed in **Table 6**.

Table 6. Challenges and Future Directions in WBS-AMR Surveillance

Challenge Category	Specific Challenge	Impact on WBS-AMR Surveillance	Current Limitation	Recommended Future Direction
Standardization	Lack of harmonized sampling and analytical protocols	Poor inter-study comparability and inconsistent ARG quantification	Different sampling volumes, extraction methods, sequencing depths, and normalization strategies across studies	Development of globally accepted SOPs and standardized surveillance guidelines
Environmental Variability	Seasonal fluctuations in ARG abundance	Temporal inconsistency in surveillance data due to infection waves and antibiotic usage trends	Most studies rely on short-term or cross-sectional datasets	Longitudinal multi-season monitoring with temporal normalization models
Wastewater Heterogeneity	Variability in wastewater composition (organic load, pH, inhibitors, industrial discharge)	Reduced sensitivity and inconsistent biosensor/ qPCR performance	Complex wastewater matrices interfere with nucleic acid extraction and electrochemical sensing	Matrix-specific calibration and adaptive pre-processing strategies
Dilution Effects	Rainfall events and hydraulic fluctuations alter ARG concentration	False reduction or inflation of resistance gene abundance	Limited incorporation of wastewater flow normalization	Integration of flow-adjusted and population-normalized measurements
Geographic Variability	Differences in sewer infrastructure and sanitation systems across regions	Difficulty comparing AMR burden across countries and	Existing protocols are often optimized for high-resource urban settings	Development of region-specific surveillance optimization frameworks

		communities		
Sensitivity Issues	Low abundance ARGs may remain undetected in complex samples	Reduced early-warning capability for emerging resistance markers	Signal interference and low target recovery in environmental samples	Improved signal amplification, nanomaterial-assisted bio-sensing, and enhanced extraction efficiency
Biosensor Validation	Limited field-scale validation of biosensor platforms	Uncertain reliability under real wastewater conditions	Most biosensor studies remain laboratory-based	Multi-site field validation studies and benchmarking against sequencing methods
Multiplex Detection	Simultaneous detection of multiple ARGs remains technically challenging	Reduced surveillance breadth in rapid detection systems	Cross-reactivity and limited multiplexing capacity	Advanced microfluidic multiplex platforms and AI-assisted signal differentiation
Data Integration	Integration of metagenomics, qPCR, biosensor, and epidemiological datasets	Fragmented interpretation of AMR trends	Lack of unified analytical pipelines	AI-driven integrated surveillance dashboards and cloud-based analytics
Computational Burden	Large-scale Metagenomic datasets require advanced computational resources	Delayed analysis and reduced accessibility in low-resource settings	High dependence on bioinformatics expertise	Automated pipelines and lightweight AI-assisted analytical tools
Infrastructure Requirements	Dependence on centralized laboratory facilities	Limited scalability in remote or resource-limited settings	Sequencing infrastructure remains expensive and inaccessible	Portable decentralized biosensor-enabled surveillance systems
Calibration and Maintenance	Frequent biosensor calibration required for accurate detection	Signal drift and variability over time	Lack of robust long-term environmental stability data	Self-calibrating sensor systems and standardized maintenance schedules
Ethical and Privacy Concerns	Population-level wastewater surveillance may raise ethical concerns	Potential misuse or stigmatization of monitored communities	Limited regulatory frameworks for WBS-AMR data governance	Ethical oversight frameworks and anonymized surveillance policies
Public Health Integration	Weak integration between environmental and clinical surveillance systems	Reduced translational impact of WBS findings	Environmental datasets often remain disconnected from healthcare decision-making	Establishment of One Health integrated AMR surveillance networks
Cost and Scalability	High costs associated with sequencing and advanced analytics	Limited adoption in low- and middle-income countries	Long-term operational sustainability challenges	Low-cost portable biosensors and scalable decentralized monitoring systems
Real-Time Surveillance	Delayed turnaround time in sequencing-based surveillance	Reduced outbreak response capability	Current methods often require days for analysis	Development of rapid electrochemical Lab-on-a-Chip platforms for near real-time detection

IX. CONCLUSION

This WBS would become an efficient means of analyzing the spread of AMR in different ecological and health settings through environmental epidemiological approaches. Despite modern tools like HT-qPCR and metagenomics have already become important components of the WBS framework, the requirement to provide all necessary laboratory facilities is one of the crucial difficulties that needs to be overcome in order to respond rapidly to any problem. The novel approach presented herein, is the utilization of next generation surveillance and biosensors technologies together with application of AI methods for AMR surveillance. Implementation of fast tests, hotspot analysis, longitudinal profiling of Resistome intends to move beyond retrospective surveillance. Future research directions would include obtaining consensus regarding sampling standardization, environmental biosensors testing, multi-site testing, among others. The

end-to-end processing pipeline, from mechanical filtration to downstream predictive mapping, is summarized visually in **Figure 4**.

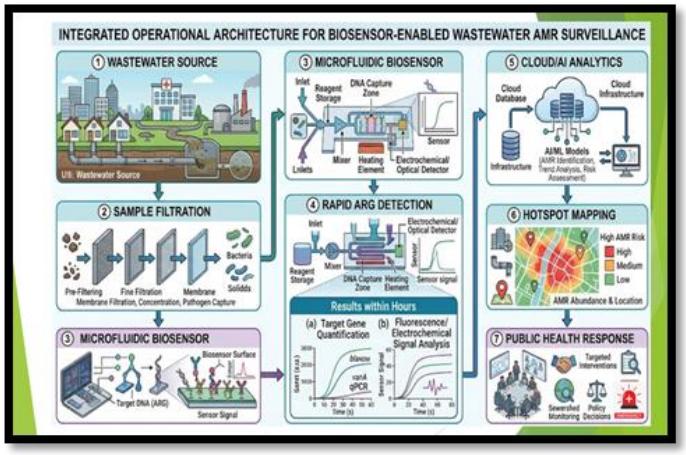

Figure 4. Summary of the Biosensor-Wastewater AMR Pipeline**:** Schematic diagram detailing the technological framework for real-time epidemiological tracking. The process begins with automated sampling from diverse urban sectors, followed by multi-stage membrane filtration to isolate bacterial targets. On-chip microfluidic biosensors execute rapid DNA capture and electrochemical/optical signal amplification, yielding quantitative ARG profiles (e.g., target gene replication curves) within hours. Downstream data is securely transmitted to cloud infrastructure for machine learning-driven risk assessment, generating predictive hotspot maps to guide targeted epidemiological interventions and policy-making.

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