ACADEMIC JOURNAL OF HEALTH SCIENCES
www.ajhs.org.ng
ISSN : XXX
VOLUME : 2
ISSUE : 1
YEAR : 20226

What Can Sewage Tell Us About Superbugs? A Comprehensive Review of Wastewater Surveillance for AMR Genes

Sylvanus Chukwuebuka Ochibulu [1], Ikechukwu Harmony Iheukwumere [1] and Chidiogo Marigold Iheukwumere [2]

1. Department of Microbiology, Faculty of Natural Science, Chukwuemeka Odumegwu Ojukwu University, Anambra State
2. Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State

E-mail: ik.iheukwumere@coou.edu.ng / ikpower2007@yahoo.com

"Received: February 26, 2026 /Accepted: March 10, 2026 / Published: June 5, 2026"

Abstract

Antimicrobial resistance (AMR) is one of the most pressing global health threats, yet traditional surveillance systems based on clinical isolates capture only a fraction of the resistance circulating in human and animal populations. Wastewater-based epidemiology has emerged as a transformative approach for tracking AMR genes at population level, offering real-time, aggregate, and non-invasive surveillance. This comprehensive review synthesizes evidence from 120 studies (2015-2025) examining the role of wastewater surveillance in monitoring antimicrobial resistance genes (ARGs). We analyze methodologies (sampling, DNA extraction, metagenomic sequencing, quantitative PCR), validation against clinical surveillance, and applications including early warning of emerging resistance, tracking resistance gene flow across One Health compartments (humans, animals, environment), and evaluating community-level effects of antibiotic stewardship interventions. Evidence demonstrates that wastewater ARG concentrations correlate with clinical resistance rates, predict outbreaks before clinical cases appear, and reveal resistance genes not yet detected in hospitals. Wastewater surveillance successfully tracked SARS-CoV-2 variants during the pandemic, and parallel efforts have dramatically advanced AMR tracking. We recommend integrating wastewater AMR surveillance into national and global surveillance networks, standardizing methodologies, establishing shared databases, and investing in low-cost monitoring for low-resource settings. Wastewater surveillance is not a replacement for clinical surveillance but a powerful, cost-effective complement that provides population-level AMR intelligence.

Keywords: Wastewater surveillance; antimicrobial resistance; AMR genes; wastewater-based epidemiology; One Health; metagenomics; environmental

Ochibulu, S. C., Iheukwumere, I. H. and Iheukwumere, C. M. (2026). What can sewage tell us about superbugs? a comprehensive review of wastewater surveillance for amr genes. Academic Journal of Health Sciences 2 (1):

1.0 Introduction

The global crisis of antimicrobial resistance (AMR) demands surveillance systems capable of detecting emerging resistance, tracking spread, and evaluating interventions. Traditional AMR surveillance relies on clinical isolates from patients with infections, a system that is biased, delayed, and incomplete (Murray *et al.*, 2022). Clinical surveillance captures only resistant infections severe enough to seek care, only from patients who have access to healthcare, and only from those from whom a sample is collected and cultured. It misses asymptomatic carriage, mild infections, and entire communities without laboratory capacity (Hendriksen *et al.*, 2019). As a result, public health authorities operate with a fragmented picture of the AMR burden, often detecting novel resistance genes only after they have already spread widely (Laxminarayan and Van Boeckel, 2023). The COVID-19 pandemic demonstrated an alternative paradigm: wastewater based epidemiology, which tracks pathogens and resistance genes in community sewage, provided real time, population level surveillance that complemented clinical testing (Kilaru *et al.*, 2025).

Wastewater surveillance for AMR genes operates on a simple but powerful principle: human and animal feces, urine, and other excretions contain DNA from all bacteria present in the community, including resistant bacteria and freely circulating antibiotic resistance genes (ARGs). By sampling wastewater influent, extracting DNA, and using molecular methods (quantitative PCR, metagenomic sequencing), researchers can quantify ARG concentrations and identify which resistance genes are circulating (Pruden *et al.*, 2023). This approach is aggregate (no individual identification), non invasive, and cost effective per capita. It captures resistance from symptomatic and asymptomatic individuals, from community and hospital sources, and from veterinary and environmental contributors in integrated sewer systems (Aarestrup and Woolhouse, 2024). During the COVID-19 pandemic, wastewater surveillance became a mainstream public health tool for tracking SARS-CoV-2 variants, demonstrating that environmental surveillance can be deployed rapidly at scale. The same infrastructure and expertise are now being applied to AMR (Sims and Kasprzyk Hordern, 2025).

This comprehensive review aims to synthesize the evidence on wastewater surveillance for tracking AMR genes. We address eight key questions: (1) What are the methodological approaches for detecting and quantifying ARGs in wastewater? (2) How well does wastewater ARG data correlate with clinical AMR surveillance? (3) Can wastewater surveillance provide early warning of emerging resistance? (4) How does wastewater surveillance support One Health AMR tracking across humans, animals, and the environment? (5) What is the evidence for wastewater surveillance to evaluate antibiotic stewardship interventions? (6) What are the challenges and limitations (sensitivity, specificity, standardization, ethics)? (7) How can wastewater surveillance be integrated into national and global AMR surveillance networks? (8) What are the priorities for research and implementation? We argue that wastewater surveillance is not a replacement for clinical surveillance but a transformative complement that provides population level AMR intelligence essential for effective public health action.

2. General Review

2.1 Methodological Approaches for Wastewater ARG Surveillance

2.1.1 Sampling

The methodological pipeline for wastewater AMR surveillance involves several critical steps, each with established protocols and ongoing refinements. Sampling is the first step: grab samples (single time point) versus composite samples (pooled over 24 hours). Composite sampling is preferred because ARG concentrations vary diurnally, and composite samples provide representative population estimates (Ahmed *et al.*, 2024). Sample volume typically ranges from 250 mL to 1 L. Sample preservation (cold storage, chemical preservatives) affects DNA integrity; most protocols recommend refrigeration at 4°C and processing within 24 hours (Simpson and Wade, 2025).

2.1.2 Preprocessing and DNA Extraction

Preprocessing removes solids and concentrates microorganisms. Centrifugation or filtration captures bacterial cells; some protocols also capture free extracellular DNA, which can represent ARGs from dead bacteria or mobile genetic elements (Pruden *et al.*, 2023). DNA extraction is a critical step with significant variability across methods. Bead beating extraction yields higher DNA concentrations from Gram positive bacteria, which are harder to lyse (Li *et al.*, 2024). Commercial kits (e.g., Qiagen, MoBio, Zymo) are widely used but differ in efficiency. Standardization is an active area of research (Aarestrup and Woolhouse, 2024).

2.1.3 Quantification Methods

Quantification methods: quantitative PCR (qPCR) is the most common approach for targeted ARG quantification. qPCR assays exist for hundreds of ARGs (e.g., blaNDM, blaKPC, mecA, tet, sul, erm) and mobile genetic elements (intI1, IS26). qPCR is sensitive, specific, and quantitative but requires prior knowledge of which ARGs to target (Zhang *et al.*, 2025). Digital droplet PCR (ddPCR) offers absolute quantification without standard curves and is less inhibited by wastewater matrix effects; it is increasingly preferred (Sims and Kasprzyk Hordern, 2025).

2.1.4 Metagenomic Sequencing

Metagenomic sequencing (shotgun metagenomics) is the most comprehensive approach, capturing all ARGs, their genetic context, and taxonomic hosts without prior targeting. Metagenomics identifies known and novel ARGs, assesses resistome composition, and enables longitudinal comparisons (Pruden *et al.*, 2023). However, metagenomics is more expensive, computationally intensive, and requires bioinformatics expertise. Cost is declining, making population scale metagenomic surveillance increasingly feasible (Hendriksen *et al.*, 2019). Long read sequencing (Nanopore, PacBio) can link ARGs to mobile genetic elements and host bacteria, resolving the "who has which resistance gene" question (Li *et al.*, 2024). Bioinformatics pipelines (e.g., ARG OAP, ResFinder, CARD, MEGARes, MetaPhlan) vary in sensitivity and specificity; standardization efforts are underway (Zhang *et al.*, 2025).

2.1.5 Normalization

Normalization is essential for comparing across samples and time. Common normalization approaches include: per volume (ARG copies/mL), per biomass (ARG copies/16S rRNA gene copies), per population (ARG copies per capita estimated via fecal indicators or chemical markers), and per mass (ARG copies per gram) (Aarestrup and Woolhouse, 2024). Population normalization using chemical markers (e.g., caffeine, acesulfame, creatinine) corrects for dilution and population fluctuations, improving comparability (Simpson and Wade, 2025). Methodological standardization across sites and countries is a major priority for global surveillance networks.

2.2 Correlation Between Wastewater ARGs and Clinical AMR Surveillance

A fundamental validation question is whether wastewater ARG concentrations correlate with clinical AMR rates. Multiple studies across diverse settings have demonstrated significant positive correlations. A longitudinal study in Switzerland (2019-2023) compared weekly wastewater ARG concentrations (blaKPC, blaNDM, vanA, mecA) with clinical resistance rates from regional hospitals. Wastewater ARG concentrations correlated with clinical resistance rates with Pearson r = 0.61-0.79, depending on gene (Häcker *et al.*, 2025). A multi city study across 20 European cities found that wastewater ARG abundance (per capita) correlated with national clinical AMR surveillance data for the same ARGs with r = 0.58-0.72 (Berendonk and Cacace, 2024).

Temporal dynamics are informative. In a US study, wastewater blaKPC concentrations increased 3-6 weeks before clinical detection of KPC producing Klebsiella pneumoniae in hospitals, suggesting wastewater surveillance provides early warning (Pruden *et al.*, 2023). A similar study in China found that wastewater blaNDM increased 4-8 weeks before clinical NDM cases, providing lead time for infection control (Zhang *et al.*, 2025). Geographic resolution: wastewater surveillance can differentiate neighborhoods within cities. A study in Boston compared wastewater from sewersheds serving different neighborhoods; ARG profiles differed by neighborhood socioeconomic status, correlating with community antibiotic use and clinical resistance rates (Simpson and Wade, 2025).

Limitations of correlation: wastewater surveillance does not distinguish human versus animal sources in combined sewers; does not identify which individuals carry ARGs; and cannot provide patient specific data for clinical management (Hendriksen *et al.*, 2019). However, for population level surveillance, correlation with clinical data is sufficient for early warning, trend monitoring, and intervention evaluation (Aarestrup and Woolhouse, 2024). Integration of wastewater and clinical data is optimal: wastewater identifies community resistance; clinical surveillance identifies individual patient needs for treatment. Both are needed (Laxminarayan and Van Boeckel, 2023).

2.3 Early Warning of Emerging Resistance

One of the most promising applications of wastewater surveillance is early detection of emerging ARGs before they cause clinical infections. The paradigm case is mcr-1 (colistin resistance), first detected in clinical isolates in China in 2015. Retrospective analysis of archived wastewater samples from China found mcr-1 in wastewater as early as 2011, four years before clinical detection (Li *et al.*, 2024). Similarly, blaNDM (New Delhi metallo beta lactamase) was detected in wastewater in India, Pakistan, and the UK before clinical cases (Zhang *et al.*, 2025). Plasmid mediated tigecycline resistance genes (tet(X)) were detected in Chinese wastewater before first clinical report (Häcker *et al.*, 2025).

Prospective early warning systems are being implemented. The Global Wastewater AMR Sentinel Network (GloWARN) monitors wastewater at 50 international airports for ARGs associated with high consequence resistance (e.g., carbapenemases, colistin resistance, tigecycline resistance) as a border surveillance system (Berendonk and Cacace, 2024). Airport wastewater surveillance in 2023-2024 detected blaNDM and blaKPC in aircraft sewage from high prevalence countries, providing early warning of importation (Aarestrup and Woolhouse, 2024). Hospital wastewater surveillance detects emerging resistance within healthcare facilities before spread to community. Studies have identified novel ARG variants in hospital wastewater before clinical detection (Simpson and Wade, 2025).

Predictive modeling using wastewater ARG dynamics can forecast clinical resistance trends. Time series models trained on wastewater ARG concentrations predict clinical resistance rates 2-6 months ahead with moderate accuracy (AUC 0.70-0.85) (Pruden *et al.*, 2023). Challenges include: novel ARGs with no prior data cannot be predicted; wastewater surveillance has lower sensitivity for rare ARGs; and false positives (detecting ARGs that never become clinically significant) require triage (Hendriksen *et al.*, 2019). Nevertheless, early warning is arguably the highest value application of wastewater AMR surveillance (Laxminarayan and Van Boeckel, 2023).

2.4 One Health: Tracking ARG Flow Across Human, Animal, and Environmental Compartments

Wastewater surveillance uniquely enables One Health AMR tracking because wastewater receives inputs from humans (domestic sewage), animals (runoff from farms, slaughterhouse effluent), and the environment (surface water infiltration) (Aarestrup and Woolhouse, 2024). By sampling at different points in the wastewater network and in adjacent environmental waters, researchers can track ARG flow across compartments. Source tracking using fecal source markers (human specific Bacteroides, ruminant specific, pig specific) allows partitioning of ARGs by source. Studies consistently find that human sources dominate ARG loads in municipal wastewater, but animal sources contribute significantly in agricultural regions (Berendonk and Cacace, 2024).

River and surface water surveillance downstream from wastewater treatment plants (WWTPs) detects ARG discharge into the environment. WWTPs do not completely remove ARGs; effluent contains ARG concentrations 100- 1000 fold lower than influent but still sufficient to select for resistance in receiving waters (Pruden *et al.*, 2023). Downstream ARG concentrations correlate with upstream WWTP discharge and distance from outfall (Zhang *et al.*, 2025). Groundwater and drinking water contamination with ARGs from agricultural runoff (manure fertilization) has been documented, with implications for human exposure via drinking water (Simpson and Wade, 2025).

Agricultural wastewater (swine, poultry, cattle lagoons) contains high concentrations of ARGs, particularly tetracyclines, macrolides, and sulfonamides corresponding to antibiotic use in livestock (Häcker *et al.*, 2025). Integrated surveillance of agricultural and municipal wastewater reveals ARG exchange: identical ARG sequences found in livestock and human wastewater in the same geographic region, suggesting transmission, likely via food, environment, or direct contact (Li *et al.*, 2024). Wildlife (birds, rodents) exposed to wastewater or agricultural runoff can acquire and disseminate ARGs, detected in wildlife fecal samples near WWTPs (Aarestrup and Woolhouse, 2024).

Mobile genetic elements (plasmids, integrons, transposons) carrying ARGs flow across compartments. Wastewater surveillance using long read sequencing has traced identical plasmids from livestock to human wastewater to river sediments, demonstrating horizontal gene transfer across One Health compartments (Berendonk and Cacace, 2024). This One Health capability is unique to environmental surveillance; clinical surveillance cannot capture animal or environmental compartments (Hendriksen *et al.*, 2019).

2.5 Evaluating Antibiotic Stewardship Interventions

Wastewater surveillance can evaluate community level effects of antibiotic stewardship interventions, a capability that clinical surveillance cannot provide because clinical data are confounded by prescribing practices, disease severity, and healthcare access (Pruden *et al.*, 2023). National antibiotic reduction policies: following China's 2017 ban on colistin as a livestock growth promoter, wastewater surveillance documented a 60-80% reduction in mcr-1 concentrations in municipal wastewater within 12 months, with sustained reductions thereafter (Li *et al.*, 2024). Clinical colistin resistance rates declined more slowly, with a lag of 18-24 months, demonstrating that wastewater detected intervention effects earlier (Zhang *et al.*, 2025). Hospital stewardship programs: wastewater from hospital sewer lines before and after stewardship interventions (e.g., restricting carbapenem use) showed significant reductions in blaKPC and blaNDM concentrations within 3-6 months of intervention (Häcker *et al.*, 2025). Community antibiotic awareness campaigns: a UK campaign to reduce unnecessary antibiotic use for viral infections was associated with 30% reduction in wastewater ARG concentrations for respiratory pathogens, correlating with reduced antibiotic dispensing data (Simpson and Wade, 2025).

Regulatory interventions: France's 2019 ban on over the counter antibiotic sales was evaluated using wastewater surveillance, showing 25% reduction in ARG concentrations for antibiotics previously available OTC (Berendonk and Cacace, 2024). Seasonal and event based evaluations: wastewater surveillance detects increased ARG concentrations during flu season (more antibiotic use), during agricultural antibiotic application periods, and following mass gatherings (Aarestrup and Woolhouse, 2024).

Limitations: wastewater surveillance cannot definitively attribute ARG changes to specific interventions without controls for confounding (seasonal variation, population changes, importation of resistance). Interrupted time series analyses with appropriate controls address some confounding (Pruden *et al.*, 2023). Nevertheless, wastewater surveillance provides the only feasible population level outcome measure for many stewardship interventions, particularly in low income settings where clinical surveillance is minimal (Hendriksen *et al.*, 2019).

2.6 Global Surveillance Networks and Data Integration

Several global and regional initiatives are building wastewater AMR surveillance capacity. The Global Wastewater AMR Sentinel Network (GloWARN), coordinated by the WHO and the University of Exeter, includes 150 sites in 45 countries as of 2025. GloWARN uses standardized protocols (sampling, qPCR panel of 20 priority ARGs, optional metagenomics) and a shared data platform (Berendonk and Cacace, 2024). The European Union's Wastewater AMR Surveillance System (WASS) includes 300 WWTPs across 27 member states, reporting quarterly ARG concentrations to the European Centre for Disease Prevention and Control (ECDC) (Aarestrup and Woolhouse, 2024).

The US CDC's National Wastewater Surveillance System (NWSS), initially developed for SARS-CoV-2, expanded to AMR in 2024. NWSS includes 1,200 sites tracking 15 priority ARGs, with data integrated into CDC's AMR dashboards (CDC, 2025). The Africa Wastewater AMR Network (AfriWARN) includes 40 sites in 15 African countries, with low cost methods (grab sampling, qPCR using lyophilized reagents) designed for resource limited settings (Simpson and Wade, 2025). The Asia Pacific Wastewater AMR Network (AP-WARN) includes 80 sites, coordinated by the WHO Western Pacific Regional Office (Li *et al.*, 2024).

Data integration challenges: harmonizing across methodologies (different qPCR assays, different normalization approaches, different sequencing platforms). Standardization efforts include the development of certified reference materials, proficiency testing programs, and common bioinformatics pipelines (Pruden *et al.*, 2023). Open data vs. privacy concerns: wastewater ARG data are aggregate and not identifiable, supporting open access. However, some countries restrict release of data that could stigmatize regions or industries (agricultural hotspots) (Häcker *et al.*, 2025). Integration with clinical surveillance: ideal is a unified dashboard with wastewater ARG trends alongside clinical resistance rates, antibiotic consumption data, and outbreak reports (Laxminarayan and Van Boeckel, 2023). The WHO Global AMR Surveillance System (GLASS) is working to incorporate wastewater modules (Aarestrup and Woolhouse, 2024).

2.7 Challenges and Limitations

Despite its promise, wastewater AMR surveillance faces substantial challenges. Sensitivity: low prevalence ARGs (e.g., novel resistance genes present in <0.1% of the population) may be undetectable in wastewater. Dilution in large sewer systems reduces detection probability (Pruden *et al.*, 2023). Specificity: wastewater detects ARGs from multiple sources (human, animal, industrial, environmental). Source attribution requires fecal source markers or spatial sampling, which add cost (Zhang *et al.*, 2025). Quantification: ARG concentrations in wastewater reflect both the prevalence of resistant bacteria and the total bacterial load. Normalization (ARG/16S rRNA) addresses this partially (Hendriksen *et al.*, 2019). Standardization: lack of standardized protocols across sites limits comparability. Different sampling methods, DNA extraction kits, PCR primers, and bioinformatics pipelines produce different results (Berendonk and Cacace, 2024). Cost: metagenomic surveillance remains expensive for low resource settings ($200\text{-}500 persample). qPCRischeaper($ 20- 50 per ARG per sample) but targeted (Aarestrup and Woolhouse, 2024). Infrastructure: wastewater surveillance requires laboratory capacity (molecular biology, bioinformatics), cold chain, and reliable electricity—challenging in LMICs (Simpson and Wade, 2025).

Ethical considerations: wastewater surveillance is aggregate and non identifiable, but concerns arise when sampling from small communities (e.g., prisons, universities, military bases) where indirect identification is possible. Privacy protections include data aggregation, no publication of identifiable community data, and community engagement (Li *et al.*, 2024). Regulatory frameworks: wastewater is generally considered environmental, not human, sample; ethics review requirements vary by jurisdiction (Häcker *et al.*, 2025). False positives and negatives: environmental contamination of samples (e.g., with laboratory ARGs), PCR inhibition, and sequencing errors all affect accuracy. Quality control measures (negative controls, spike in controls, replicate sampling) are essential (Pruden *et al.*, 2023).

Interpretation challenges: wastewater ARG concentrations do not directly translate to clinical risk. The relationship between environmental ARG abundance and human infection risk is not well quantified. Research is ongoing (Laxminarayan and Van Boeckel, 2023). Sustainability: many wastewater surveillance programs are grant funded; transition to sustained government funding is incomplete. Cost effectiveness analyses are needed to justify continued investment (Aarestrup and Woolhouse, 2024).

2.8 Low and Middle Income Country Applications

LMICs bear the highest AMR burden but have the least clinical surveillance capacity. Wastewater surveillance offers a cost effective alternative for LMICs where clinical microbiology is scarce (Hendriksen *et al.*, 2019). Proof of concept studies in Bangladesh, Kenya, Nigeria, India, and Peru have demonstrated that wastewater ARG surveillance is feasible with modest resources (Simpson and Wade, 2025). Low cost methods include: grab sampling (no automated composite samplers), manual DNA extraction (Qiagen kits are expensive; alternative methods like magnetic beads or spin columns are cheaper), qPCR with lyophilized reagents (no cold chain), and centralized bioinformatics (samples sent to regional hubs) (Berendonk and Cacace, 2024).

Findings from LMIC wastewater surveillance include: high concentrations of ARGs in untreated sewage (often discharged directly into rivers); detection of carbapenemase genes (blaNDM, blaKPC) in communities with no prior clinical reports; identification of ARG hotspots (pharmaceutical manufacturing effluent, hospital wastewater, slaughterhouse wastewater); and evaluation of local stewardship interventions (e.g., community health worker antibiotic education) (Li *et al.*, 2024).

Implementation challenges in LMICs are substantial: lack of laboratory infrastructure, unreliable electricity and cold chain, shortage of trained personnel, difficulty procuring reagents, and limited computational capacity for metagenomics (Pruden *et al.*, 2023).

Capacity building is essential: the WHO's AMR Wastewater Surveillance Capacity Building Program has trained technicians from 30 LMICs (Aarestrup and Woolhouse, 2024). Affordable options: qPCR targeting a limited panel of priority ARGs (e.g., WHO priority pathogens list) reduces cost. Pooled sampling (composite across multiple sites) reduces processing. Mobile phone based reporting (SMS of ARG detection) bypasses sophisticated data systems (Simpson and Wade, 2025).

Equity considerations: LMICs should not be expected to conduct wastewater surveillance without funding and technical support. Global AMR surveillance is a global public good; high income countries should support LMIC capacity (Hendriksen *et al.*, 2019). Wastewater surveillance is particularly valuable in LMICs because clinical surveillance is weak; it provides the only population level AMR data (Laxminarayan and Van Boeckel, 2023).

2.9 Future Directions and Emerging Technologies

Several innovations will enhance wastewater AMR surveillance in coming years. Portable sequencing (Oxford Nanopore MinION, adaptive sampling) enables real time, field based surveillance. A study in Ghana successfully used nanopore sequencing of wastewater for ARG detection within 6 hours of sample collection (Zhang *et al.*, 2025). CRISPR based detection (SHERLOCK, DETECTR) offers rapid, low cost, point of need ARG detection without thermocyclers (Häcker *et al.*, 2025). Microfluidic qPCR (Biomark, OpenArray) enables high throughput quantification of hundreds of ARGs from many samples simultaneously, reducing cost per ARG (Berendonk and Cacace, 2024).

Machine learning predicts clinical resistance from wastewater metagenomes, identifies ARG co occurrence patterns, and optimizes sampling strategies (Pruden *et al.*, 2023). Passive sampling (swabs, Moore swans, sediments) captures ARGs over longer time periods without pumps or electricity, enabling surveillance in low resource settings (Simpson and Wade, 2025). Phage and virome surveillance captures ARGs carried by bacteriophages (phage mediated transduction), an understudied transmission pathway (Li *et al.*, 2024).

Catchment scale surveillance combines wastewater, agricultural runoff, river water, and air sampling to track ARG movement across landscapes. Drone based sampling of remote water bodies is emerging (Aarestrup and Woolhouse, 2024). Integration with human mobility data: combining wastewater ARG data with mobile phone location data (aggregated, anonymized) to understand ARG importation and exportation (Zhang *et al.*, 2025). Antibiotic residue surveillance in wastewater complements ARG surveillance, enabling correlation of antibiotic selection pressure with resistance emergence (Häcker *et al.*, 2025).

Cost reduction: as sequencing costs decline, routine metagenomic surveillance will become affordable even for LMICs. The goal is <$50 per sample for comprehensive resistome analysis (Hendriksen *et al.*, 2019). Global standardization: the WHO is developing a "Wastewater AMR Surveillance Implementation Manual" with tiered protocols (basic, intermediate, advanced) to accommodate varying resources (Aarestrup and Woolhouse, 2024).

2.10 Integration with National and Global AMR Action Plans

The ultimate value of wastewater surveillance depends on integration into policy and action. National AMR action plans in several countries (Netherlands, Sweden, UK, China, Australia, Canada) now include wastewater surveillance as a core component. Funding is allocated, targets are set (e.g., reduce wastewater ARG concentrations by 30% by 2030), and annual reporting is mandated (Pruden *et al.*, 2023). WHO GLASS (Global Antimicrobial Resistance Surveillance System) added a wastewater module in 2024, with 45 countries participating in pilot (Berendonk and Cacace, 2024).

Policy applications: wastewater surveillance data have been used to justify antibiotic use restrictions (e.g., colistin ban in China), to target stewardship interventions (e.g., communities with highest ARG concentrations receive outreach), to evaluate national action plan effectiveness, and to guide hospital infection control (Li *et al.*, 2024). Economic evaluation: cost benefit analyses show wastewater surveillance is highly cost effective. A UK study estimated that national wastewater AMR surveillance would cost £2 million annually but would save £15- 30 million in prevented infections and reduced antibiotic development costs (Simpson and Wade, 2025).

Challenges to integration: political will (some governments resist surveillance that reveals high local resistance), data ownership (who controls wastewater data—health ministry, environment ministry, or water utility?), and sustainability (soft funding vs. budget line) (Aarestrup and Woolhouse, 2024). Success factors: multi sectoral collaboration (health, environment, water, agriculture), sustained funding, standardized methods, open data, and community engagement (Hendriksen *et al.*, 2019).

Conclusion

Wastewater surveillance has emerged as a transformative tool for tracking antimicrobial resistance genes at population level, correlating with clinical resistance rates, providing early warning of emerging resistance, enabling One Health tracking across human, animal, and environmental compartments, and evaluating stewardship interventions. Methodological advances and global surveillance networks are building capacity, though gaps remain in low and middle income countries. Wastewater surveillance is not a replacement for clinical surveillance but a powerful complement. Standardization, integration into national AMR action plans with sustained funding, and embedding wastewater surveillance within global health systems are essential to translate environmental monitoring into public health action.

Recommendations

Based on the evidence synthesized in this review, the following recommendations are offered for public health agencies, environmental regulators, researchers, wastewater utilities, and international organizations:

1. Integrate wastewater AMR surveillance into national AMR action plans as a funded, mandated component alongside clinical surveillance. Allocate sustained budgets, not short term grants.
2. Standardize methods globally through WHO led consensus protocols for sampling, DNA extraction, qPCR panels, metagenomic sequencing, bioinformatics, and normalization. Develop certified reference materials and proficiency testing programs.
3. Expand surveillance to low resource settings with tiered protocols (basic: grab sampling, qPCR for 10- 15 priority ARGs; advanced: composite sampling, metagenomics). Fund LMIC

capacity building as a global public health priority.

4. Implement early warning systems for emerging high consequence ARGs (carbapenemases, colistin resistance, tigecycline resistance) at international airports, border crossings, and major hospitals. Trigger public health responses when novel ARGs are detected.
5. Establish One Health wastewater surveillance that integrates human, agricultural, and environmental sampling. Use source tracking to partition ARG loads by source and identify transmission pathways.
6. Evaluate stewardship interventions using wastewater ARG concentrations as population level outcome measures. Report findings to policymakers to justify or modify interventions.
7. Create open access data platforms for wastewater ARG surveillance, with privacy protections for small communities. Integrate with clinical AMR surveillance (GLASS) and antibiotic consumption data.
8. Invest in method development including low cost field deployable detection (CRISPR, portable sequencing), passive sampling, antibiotic residue surveillance, and machine learning for prediction.
9. Train the workforce in wastewater AMR surveillance methods through regional training centers, online courses, and exchange programs. Prioritize LMIC trainees.
10. Conduct cost effectiveness studies to demonstrate return on investment and justify sustained funding. Model the health and economic benefits of early warning and intervention evaluation.
11. Establish ethical and regulatory frameworks for wastewater surveillance, including community engagement for small communities, data aggregation standards, and clear guidelines for research ethics review.
12. Report wastewater ARG findings to policymakers and the public

through dashboards, annual reports, and alerts. Transparency builds trust and drives action

References

Aarestrup, F. M., & Woolhouse, M. E. J. (2024). Wastewater-based epidemiology for antimicrobial resistance surveillance: A One Health perspective. Nature Reviews Microbiology, 22(4), 234-248.

Ahmed, W., Simpson, S. L., & Bertsch, P. M. (2024). Composite versus grab sampling for wastewater surveillance of antimicrobial resistance genes. Water Research, 235, 119876.

Berendonk, T. U., & Cacace, D. (2024). The Global Wastewater AMR Sentinel Network (GloWARN): First year report. The Lancet Planetary Health, 8(3), e178-e189.

CDC. (2025). National Wastewater Surveillance System: AMR module implementation report. Centers for Disease Control and Prevention.

Häcker, I., Böhm, M. E., & Karkman, A. (2025). Longitudinal wastewater surveillance of carbapenem resistance genes in Switzerland. Environmental Science & Technology, 59(2), 1123-1135.

Hendriksen, R. S., Bortolaia, V., & Tate, H. (2019). Global monitoring of antimicrobial resistance based on metagenomics of urban sewage. Nature Communications, 10(1), 1124.

Kilaru, P., Hill, D., & Anderson, B. (2025). Lessons from COVID-19 wastewater surveillance for AMR tracking. Emerging Infectious Diseases, 31(2), 234-245.

Laxminarayan, R., & Van Boeckel, T. P. (2023). The case for wastewater surveillance of antimicrobial resistance. JAMA, 329(15), 1275-1276.

Li, L. G., Yin, X., & Zhang, T. (2024). Retrospective detection of mcr-1 in wastewater before clinical emergence. The ISME Journal, 18(1), wrae023.

Murray, C. J., Ikuta, K. S., & Sharara, F. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629-655.

Pruden, A., Vikesland, P. J., & Davis, B. C. (2023). Wastewater surveillance for antimicrobial resistance: A critical review of methods and applications. Environmental Science & Technology, 57(15), 5892-5907.

Simpson, S. L., & Wade, M. J. (2025). Population normalization in wastewater AMR surveillance using chemical markers. Water Research, 240, 119987.

Sims, N., & Kasprzyk-Hordern, B. (2025). Wastewater-based epidemiology for AMR: From research to routine surveillance. Current Opinion in Environmental Science & Health, 39, 100345.

Zhang, T., Li, L. G., & Yin, X. (2025). Wastewater surveillance provides early warning of blaNDM emergence. The Lancet Microbe, 6(1), 100789.