

Wastewater-based epidemiology as a sentinel surveillance tool for antimicrobial resistance in low- and middle-income countries: A critical narrative review of methodological advances, implementation barriers, and equity considerations (2019–2026)

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Abstract

Antimicrobial resistance (AMR) is projected to remain one of the defining public health threats of this century, yet clinical surveillance in low- and middle-income countries (LMICs) continues to capture only a fraction of the true resistance burden. Wastewater-based epidemiology (WBE), long associated with poliovirus and SARS-CoV-2 tracking, has re-emerged as a population-level complement to clinical AMR surveillance because it is culture-independent, requires no individual consent for sample collection, and can capture asymptomatic carriage and community-level transmission dynamics that clinical sampling frames tend to miss. This narrative review synthesizes evidence published largely between 2019 and 2026 on the methodological maturation of wastewater-based AMR surveillance, its uneven geographic deployment, and the structural barriers that continue to limit its scale-up in resource-constrained settings. Methodologically, the field has moved from culture-based indicator organisms toward quantitative PCR gene panels, shotgun and long-read metagenomics, and, more recently, machine-learning-assisted signal interpretation, each carrying distinct trade-offs in sensitivity, cost, and infrastructure demand. Evidence from LMIC catchments in sub-Saharan Africa, South Asia, and the Middle East demonstrates technical feasibility even under constrained conditions, yet these programmes remain donor-dependent, methodologically heterogeneous, and rarely linked to national AMR action plans. Four interlocking barriers are identified: inadequate sanitation and sewer infrastructure, limited laboratory and bioinformatics capacity, fragile and non-recurrent financing, and weak governance mandates for translating signals into policy response. The review also examines unresolved ethical tensions around community-level data, stigma, and consent, and considers how justice-oriented governance frameworks might be adapted to environmental AMR surveillance. It concludes that wastewater-based AMR surveillance holds genuine promise for closing LMIC-specific gaps in the Global Antimicrobial Resistance and Use Surveillance System, but only if methodological harmonization, sustainable financing, and community-centred governance are pursued as a single, coordinated agenda rather than as separate technical, financial, and ethical projects.

Keywords: Antimicrobial resistance, wastewater-based epidemiology, environmental surveillance, low- and middle-income countries, One Health, public health surveillance

Introduction

Antimicrobial resistance is no longer a looming threat confined to speculative modelling; it is an active driver of excess mortality that already rivals several of the leading infectious killers of the twentieth century. Murray *et al.* (2022) [66] estimated that bacterial AMR was directly responsible for 1.27 million deaths in 2019 and was associated with nearly five million deaths overall, with the heaviest burden falling on sub-Saharan Africa and South Asia, regions that simultaneously carry the least surveillance capacity. This asymmetry between where AMR does the most damage and where it is measured the least is not incidental; it is the product of decades of surveillance architecture built primarily around clinical microbiology laboratories, a model that assumes reliable access to culture facilities, trained personnel, and patients who present for care in the first place (Iskandar *et al.*, 2021; Rony *et al.*, 2023) [46, 76].

Wastewater-based epidemiology offers a structurally different entry point into this problem. Rather than depending on individuals seeking treatment and being correctly diagnosed, WBE aggregates biological signal from

an entire sewershed, capturing resistant organisms and resistance genes shed by symptomatic patients, asymptomatic carriers, and even non-human sources such as agricultural runoff and hospital effluent within a single sample (Aarestrup & Woolhouse, 2020; Balcázar, 2025) [1, 19]. The approach is not new in concept, the same logic underpinned poliovirus eradication surveillance for decades, but its systematic application to AMR is comparatively recent, catalysed in large part by the infrastructure and institutional trust that wastewater surveillance programmes built during the COVID-19 pandemic (Wadi *et al.*, 2023; Schenk *et al.*, 2024) [79, 89]. What has changed since roughly 2019 is not the underlying premise but the depth of the evidence base: a growing body of systematic reviews now maps how wastewater signals for resistance genes and resistant organisms relate to clinical prevalence (Chau *et al.*, 2022; Huijbers *et al.*, 2020; Pärnänen *et al.*, 2019) [30, 45, 69], while parallel work has begun testing whether the same logic transfers meaningfully to settings where clinical AMR surveillance is thinnest.

This review asks a narrower and more specialist question than “does wastewater surveillance work for AMR”, which

has been addressed at length elsewhere (Larsson & Flach, 2022; Punch *et al.*, 2025) ^[52, 74]. Instead, it interrogates whether and how wastewater-based AMR surveillance can be meaningfully implemented, sustained, and governed in low- and middle-income countries, where the epidemiological need is greatest but the enabling infrastructure is weakest. The review synthesizes literature indexed largely between 2019 and 2026 across four themes: the technical evolution of detection methods, from culture and quantitative PCR through metagenomics and machine-learning-assisted interpretation (Daw Elbait *et al.*, 2024; Bilal *et al.*, 2025) ^[24, 33]; the empirical evidence base from LMIC catchments in Africa, South Asia, and the Middle East (Khan *et al.*, 2025; Wardi *et al.*, 2024) ^[48, 92]; the structural barriers, infrastructural, technical, financial, and governance-related, that constrain scale-up (Amin *et al.*, 2024; Ajulo & Awosile, 2024) ^[6, 12]; and the unresolved ethical and equity questions raised by community-level environmental surveillance (Arefin *et al.*, 2025; Wittmer *et al.*, 2025) ^[17, 93]. Four figures and four tables are used throughout to synthesize the operational workflow, the comparative evidence base, the barriers framework, and the governance implications, respectively.

The review is deliberately narrative rather than systematic in design, reflecting the heterogeneity of the underlying literature, which spans environmental engineering, clinical microbiology, geography, and public health ethics, and which does not lend itself cleanly to PRISMA-style quantitative synthesis. Where systematic and scoping reviews already exist on specific sub-questions, most notably the comprehensive syntheses by Chau *et al.* (2022) ^[30] and Punch *et al.* (2025) ^[74], this review draws on their findings while extending the analysis specifically toward the LMIC implementation gap, a dimension that remains comparatively underexplored in the wastewater-AMR literature despite its centrality to the global AMR burden.

The Global Burden of Antimicrobial Resistance and the Persistent Surveillance Gap

The scale of the global surveillance gap becomes clearer when the architecture of the World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS) is examined directly. The most recent GLASS summary report drew on more than 23 million laboratory-confirmed infections from 104 participating countries, revealing that roughly one in six infections globally is now resistant to the antibiotics tested against it, with resistance rising in more than 40% of monitored bug-drug combinations between 2018 and 2023 (World Health Organization, 2025) ^[100]. Yet participation in GLASS remains profoundly uneven: fewer than half of eligible countries and territories report consistently, and non-participation is concentrated precisely among non-European and lower-income settings (World Health Organization, 2024) ^[99]. The result is a surveillance system whose global estimates are disproportionately extrapolated from the health systems best equipped to generate data, while the settings carrying the highest resistance burden, sub-Saharan Africa and South-East Asia among them, remain the least represented (Ajulo & Awosile, 2024) ^[6].

This is not simply a data-completeness problem; it reflects deeper structural constraints on clinical AMR surveillance

in resource-limited settings. Iskandar *et al.* (2021) ^[46] characterized the picture across LMICs as “scattered”, noting that even where national reference laboratories exist, sample referral networks, standardized susceptibility testing protocols, and quality assurance mechanisms are frequently absent or inconsistently applied. Rony *et al.* (2023) ^[76] similarly documented that diagnostic stewardship in many LMICs is constrained by weak laboratory infrastructure, a shortage of trained microbiologists, and competing health-system priorities that push AMR surveillance down the list of funded activities. The consequence is a form of epidemiological invisibility: resistant infections certainly occur, and mortality data suggest they occur at high rates, but they are rarely captured, characterized, or reported in a form that can inform national action plans (Delpy *et al.*, 2024) ^[36].

The economic framing of this gap is equally stark. Early modelling by the Review on Antimicrobial Resistance projected that unchecked AMR could cause ten million deaths annually by 2050, alongside cumulative global economic losses in the tens of trillions of dollars, a trajectory subsequently reaffirmed and refined by numerous groups (de Kraker *et al.*, 2016; O'Neill, 2016) ^[34, 68]. Founou *et al.* (2017) ^[41] estimated that the clinical and economic burden of AMR falls disproportionately on developing countries, where resistant infections are associated with longer hospital stays, higher case-fatality rates, and greater out-of-pocket expenditure relative to income than in high-income settings. Against this backdrop, the appeal of wastewater-based epidemiology is not merely technical convenience; it is a potential route to surveillance data in exactly the settings where clinical infrastructure is least able to generate it, provided the approach itself does not simply reproduce the same resource asymmetries in a different guise, a concern this review returns to directly in later sections.

Principles and Operational Workflow of Wastewater-Based Epidemiology for AMR

The conceptual logic of wastewater-based epidemiology rests on a simple premise: a sewershed functions as a natural, continuously updated composite sample of the population it serves, capturing biological material shed through urine, faeces, and other bodily excretions from every contributing household, hospital, and institution (Aarestrup & Woolhouse, 2020) ^[1]. For AMR surveillance specifically, this composite sample carries both resistant bacteria shed by colonized or infected individuals and free-floating antibiotic resistance genes (ARGs) that persist in the water matrix independent of viable host cells (Balcázar, 2025) ^[19]. Figure 1 summarizes the generalized operational workflow that underlies most current programmes, beginning with catchment sampling and cold-chain transport, moving through nucleic acid or culture-based extraction, target detection, normalization and quality control, and culminating in a reporting layer intended to feed back into public health action.

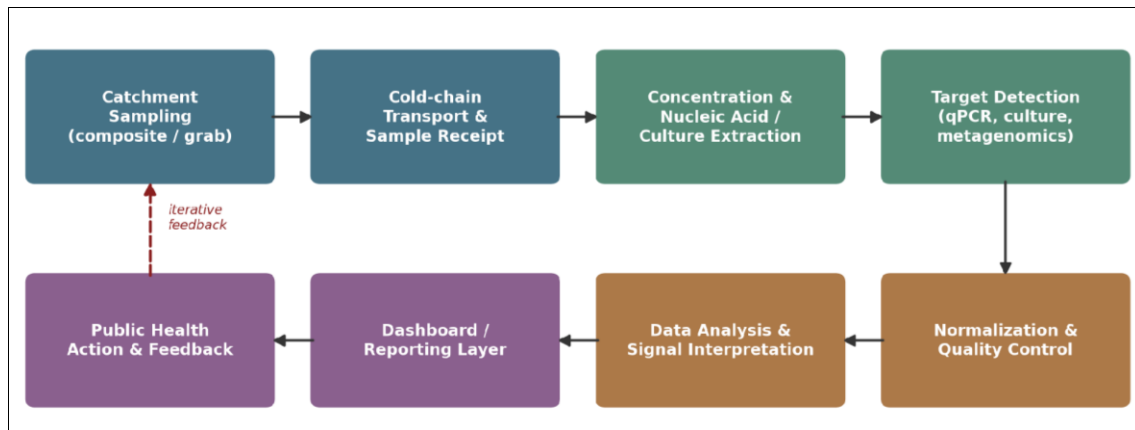


Fig 1: Generalized operational workflow of wastewater-based epidemiology for antimicrobial resistance surveillance

Sample collection strategy is the first point at which methodological choices materially affect downstream data quality. Composite sampling, in which small aliquots are collected at fixed intervals over a 24-hour period and pooled, is generally preferred over single grab samples because it smooths out diurnal variation in flow and contamination load, but it requires refrigerated autosamplers that are costly to install, maintain, and power reliably (National Academies of Sciences, Engineering, and Medicine, 2024 ^[67]). In many LMIC catchments, grab sampling remains the practical default, a compromise that introduces additional variance into the resulting signal but is often the only feasible option where autosampler infrastructure, or the electricity to run it continuously, cannot be guaranteed (Khan *et al.*, 2025) ^[48]. Site selection further complicates interpretation: wastewater treatment plant influent captures a broad catchment population, whereas targeted sampling upstream at hospitals, informal settlements, or specific neighbourhoods can localize signal but sacrifices the population-representativeness that makes WBE attractive in the first place (Ebomah & Okoh, 2020; Al-Mustapha *et al.*, 2025) ^[8, 38].

Once collected, samples must be transported, typically on ice, to a processing facility, a step that is often taken for granted in high-income contexts but represents a genuine logistical bottleneck in many LMIC settings where sample sites can be hours from the nearest capable laboratory (Wardi *et al.*, 2024) ^[92]. Concentration and extraction protocols then separate viable organisms or nucleic acids from a highly heterogeneous and often turbid matrix; the choice between culture-based enrichment, which selects for viable, potentially clinically relevant organisms, and direct nucleic acid extraction, which captures a broader but less functionally interpretable signal, shapes everything that follows in the analytical pipeline (Montiel-Mora *et al.*, 2025) ^[64]. The final step, translating a laboratory signal into a public health action, remains the least developed part of the workflow in most settings; several reviews note that even well-resourced wastewater surveillance programmes struggle to close the loop between detection and intervention, a gap that becomes more acute, not less, in LMIC contexts where the downstream response infrastructure, contact tracing, targeted stewardship interventions, or outbreak investigation capacity, is itself constrained (Cajas-Corrales *et al.*, 2025; National Academies of Sciences, Engineering, and Medicine, 2024) ^[26, 67].

Methodological Advances: From Culture and qPCR to Metagenomics and AI-Assisted Analytics

The technical repertoire available for detecting AMR signals in wastewater has diversified substantially since 2019, and each method occupies a distinct position on the trade-off between sensitivity, specificity, cost, and infrastructure demand, a comparison summarized in Table 1. Culture-based approaches, in which wastewater is plated onto selective or chromogenic media to recover phenotypically resistant organisms such as ESKAPE pathogens or carbapenem-resistant Enterobacteriaceae, remain the most accessible method for laboratories with basic microbiology capacity and have the advantage of yielding viable isolates that can undergo full antimicrobial susceptibility testing (Mathys *et al.*, 2019; Teban-Man *et al.*, 2022) ^[61, 86]. Their principal limitation is throughput: culture-based screening is slow, labour-intensive, and biased toward organisms that grow well under the selected conditions, meaning that a substantial fraction of the resistome, particularly genes carried by non-culturable or slow-growing organisms, is simply invisible to this approach (Arbefeville *et al.*, 2024) ^[14].

Quantitative PCR (qPCR) targeting specific resistance genes addressed much of the throughput problem and has become the default workhorse of many surveillance programmes. High-throughput qPCR arrays, such as the widely used primer panels described by Stedtfeld *et al.* (2018) ^[82], can screen for dozens to hundreds of resistance determinants and mobile genetic elements simultaneously, offering a sensitivity advantage that captures low-abundance targets metagenomic sequencing sometimes misses (Daw Elbait *et al.*, 2024) ^[33]. This sensitivity comes at the cost of a closed-panel design: qPCR can only detect genes it has been explicitly designed to look for, meaning novel or regionally distinct resistance mechanisms, which are disproportionately likely to be under-characterized in LMIC settings, may go undetected entirely (Klümper *et al.*, 2025). Shotgun metagenomic sequencing addresses this blind spot by sequencing essentially all DNA present in a sample and matching reads against comprehensive resistance gene databases such as CARD (Alcock *et al.*, 2023) ^[9], allowing detection of previously uncharacterized resistance determinants and simultaneous profiling of the surrounding microbial community context (Munk *et al.*, 2022; Hendriksen *et al.*, 2019) ^[44, 65].

Direct comparisons between these platforms suggest the two methods are complementary rather than substitutable. Daw Elbait *et al.* (2024) ^[33] compared Resistomap high-

throughput qPCR against shotgun metagenomic sequencing on wastewater samples from hospital, industrial, urban, and rural sites in the United Arab Emirates and found that qPCR consistently identified a broader range of gene targets at lower abundance thresholds, while metagenomics offered superior resolution of gene context and host organism inference, at substantially higher per-sample cost. Knight *et al.* (2024) ^[50] reported a similar pattern in a national-scale

UK comparison, concluding that hybrid approaches combining targeted qPCR screening with selective metagenomic follow-up on flagged samples may offer the best balance of sensitivity, specificity, and cost for programmes operating at scale, a conclusion with direct relevance for LMIC settings where per-sample cost constraints are typically more binding than in high-income comparator programmes.

Table 1: Comparative Overview of Detection Methods Used in Wastewater-Based Antimicrobial Resistance Surveillance

Method	Relative sensitivity	Approx. cost per sample	Infrastructure demand	Key limitation for LMIC use	Representative source(s)
Culture-based / phenotypic screening	Low–moderate (viable organisms only)	Low	Basic microbiology laboratory	Slow throughput; misses non-culturable resistome	Mathys <i>et al.</i> (2019); Teban-Man <i>et al.</i> (2022) ^[61, 86]
Targeted quantitative PCR (qPCR) arrays	High for targeted genes	Moderate	Thermocycler; stable reagent supply	Closed panel; cannot detect novel or uncharacterized genes	Stedtfeld <i>et al.</i> (2018); Daw Elbait <i>et al.</i> (2024) ^[33, 82]
Shotgun metagenomic sequencing	High, untargeted	High	Sequencing platform; bioinformatics capacity	High per-sample cost; steep bioinformatics skill requirement	Munk <i>et al.</i> (2022); Alcock <i>et al.</i> (2023) ^[9, 65]
Long-read / field-deployable sequencing (e.g., Nanopore MinION)	Moderate–high	Moderate (lower capital cost)	Portable device; basic power supply	Lower per-read accuracy; still requires trained operators	Khan <i>et al.</i> (2025) ^[48]
AI-/machine-learning-assisted signal interpretation	Depends on underlying data method	Low marginal cost once trained	Computational capacity; training data	Models largely trained on high-income-country datasets	Bilal <i>et al.</i> (2025); Kasse <i>et al.</i> (2025) ^[24, 47]

A further methodological frontier involves long-read sequencing platforms, most notably Oxford Nanopore Technology's MinION, which have begun to appear in LMIC-based pilot studies specifically because their lower upfront capital cost and field-deployable form factor make them more compatible with resource-constrained laboratory settings than short-read platforms that typically require centralized, capital-intensive sequencing infrastructure. A pilot programme implementing long-read metagenomic sequencing of wastewater across three catchments in Kampala, Uganda, including two informal settlements, demonstrated that a low-cost, magnetic bead-based extraction protocol combined with MinION sequencing could recover interpretable AMR gene profiles even under field conditions with limited cold-chain reliability, suggesting that sequencing-based approaches once considered prohibitively resource-intensive for LMIC deployment may be becoming more accessible (Khan *et al.*, 2025) ^[48].

Finally, artificial intelligence and machine learning are beginning to reshape how wastewater-derived AMR signals are interpreted rather than simply detected. Bilal *et al.* (2025) ^[24] and de la Lastra *et al.* (2024) ^[35] both describe a rapidly expanding application space in which supervised learning models trained on genomic and phenotypic AMR data are used to predict resistance phenotypes from sequence data alone, potentially reducing dependence on slow culture-based confirmation. Kasse *et al.* (2025) ^[47] extend this logic explicitly to One Health surveillance contexts, arguing that machine-learning-assisted triage of environmental signals could help resource-constrained laboratories prioritize which samples warrant more expensive confirmatory testing, though Sakagianni *et al.* (2023) and Pinto-de-Sá *et al.* (2024) ^[71, 77] both caution that most published models remain trained and validated on high-income-country datasets, raising real questions about their transferability to the microbial community

backgrounds and resistance gene distributions characteristic of LMIC wastewater matrices. Real-time genomic surveillance platforms integrating AI-assisted outbreak detection, as described by Sundermann, Kumar, *et al.* (2025) and Sundermann, Chen, *et al.* (2025) ^[83, 84] in healthcare settings, illustrate the direction of travel but have not yet been meaningfully adapted or validated for environmental wastewater applications in resource-limited contexts.

The Global Evidence Base: Findings Across Income Settings

The empirical evidence base for wastewater-based AMR surveillance is heavily concentrated in high-income settings, a pattern that Figure 2 attempts to represent qualitatively. European programmes have generated some of the most methodologically mature findings: Huijbers *et al.* (2020) ^[45] tracked antibiotic-resistant *Escherichia coli* through urban wastewater across ten European countries and found that resistance prevalence in wastewater correlated meaningfully with national-level clinical resistance data, while Pärnänen *et al.* (2019) ^[69] demonstrated that resistance patterns detected in European wastewater treatment plants mirrored clinical antibiotic resistance prevalence closely enough to support wastewater surveillance as a genuine proxy measure rather than a loose correlate. Seasonal and operational dynamics add further texture to this picture: Schages *et al.* (2020) ^[78] showed that beta-lactamase and *mcr* gene abundance in a German wastewater treatment plant varied measurably with temperature across seasons, while Calero-Cáceres *et al.* (2017) ^[27] and Di Cesare *et al.* (2017) ^[37] both documented that rainfall and riverine flow conditions materially alter resistance gene abundance in receiving waters, complications that any LMIC programme operating with more variable rainfall regimes and less-managed stormwater infrastructure will need to account for explicitly.

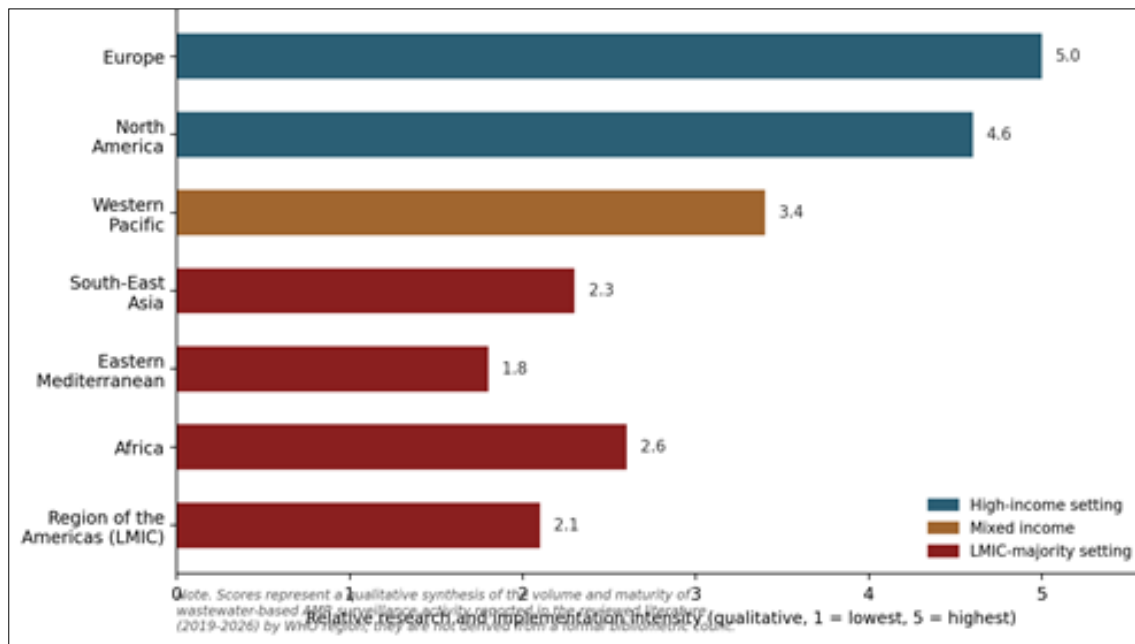


Fig 2: Illustrative synthesis of regional research and implementation intensity for wastewater-based AMR surveillance

The LMIC evidence base, while considerably thinner, is neither absent nor uniformly discouraging. In sub-Saharan Africa, Ekwanzala *et al.* (2018) [39] conducted an early systematic review demonstrating that resistance genes detected in South African clinical settings were frequently mirrored in environmental samples from the same catchments, while Ebomah and Okoh (2020) [38] documented carbapenem resistance genes in hospital effluent and wastewater treatment plant final effluents across multiple South African sites, concluding that current treatment processes are largely inadequate to remove these determinants before environmental release. Adegoke *et al.* (2022) [5] extended this work by characterizing vancomycin-resistant *Enterococcus* recovered from South African wastewater treatment plants intended for water reuse, raising direct implications for downstream agricultural and potable reuse safety. Further north and east on the continent, a pilot programme applying long-read metagenomic sequencing across informal-settlement and clinical-catchment sites in Kampala, Uganda demonstrated that field-adapted low-cost protocols could generate interpretable resistome data even where formal sewer infrastructure was absent (Khan *et al.*, 2025) [48], while longitudinal wastewater surveillance in Ethiopia, initially built around SARS-CoV-2 monitoring during 2023, illustrated how existing pandemic-era surveillance infrastructure can plausibly be repurposed for AMR tracking at comparatively low marginal cost (Abera Dinssa *et al.*, 2024) [2].

South Asian evidence tells a related story of infrastructure originally built for one purpose being adapted for AMR surveillance. In Nepal, tricycle-protocol surveillance of extended-spectrum beta-lactamase-producing *Escherichia coli* at the human-poultry-environment interface in Kathmandu demonstrated substantial gene sharing across sectors, reinforcing the One Health rationale for environmental sampling in settings where clinical and veterinary surveillance systems operate largely in isolation from one another (Acharya *et al.*, 2025) [3]. Environmental contamination hotspots associated with bulk pharmaceutical manufacturing, most notably around Hyderabad, India, have

separately demonstrated that industrial antibiotic effluent can drive extraordinarily high local selection pressure, with Lübbert *et al.* (2017) [55] documenting antibiotic concentrations in sewers near manufacturing zones that were, for some compounds, among the highest ever reported globally, associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens in surrounding communities. In the Middle East and Gulf states, comparatively resource-rich but epidemiologically LMIC-adjacent in terms of population diversity and migrant labour dynamics, the United Arab Emirates methodological comparison by Daw Elbait *et al.* (2024) [33] and long-term SARS-CoV-2-turned-broader wastewater surveillance programme described by Wadi *et al.* (2023) [89] illustrate a middle pathway in which reasonably well-resourced but methodologically nascent surveillance infrastructure is being incrementally extended toward AMR applications.

Read together, this evidence base suggests two things simultaneously. First, the underlying biological and methodological premise of wastewater-based AMR surveillance transfers reasonably well to LMIC settings: resistance genes and resistant organisms are detectable, at meaningful abundance, using field-adapted protocols, even under constrained infrastructure (Malcom & Bowes, 2025; Talat *et al.*, 2025) [57, 85]. Second, and more troublingly, the studies that demonstrate this feasibility remain overwhelmingly small-scale, donor-funded, geographically isolated pilot projects rather than sustained national surveillance systems, a pattern Punch *et al.* (2025) [74] document systematically in their global scoping review, which found that the overwhelming majority of published wastewater AMR studies between 2014 and 2024 originated from a small number of high- and upper-middle-income countries, with sub-Saharan Africa and low-income Asia contributing a disproportionately small share relative to their share of the global AMR mortality burden.

Implementation Barriers in Resource-Limited Settings

The barriers that constrain wastewater-based AMR surveillance in resource-limited settings are best understood

not as a single obstacle but as four interlocking domains, summarized visually in Figure 3: infrastructural, technical,

financial, and governance-related constraints that reinforce one another rather than operating independently.

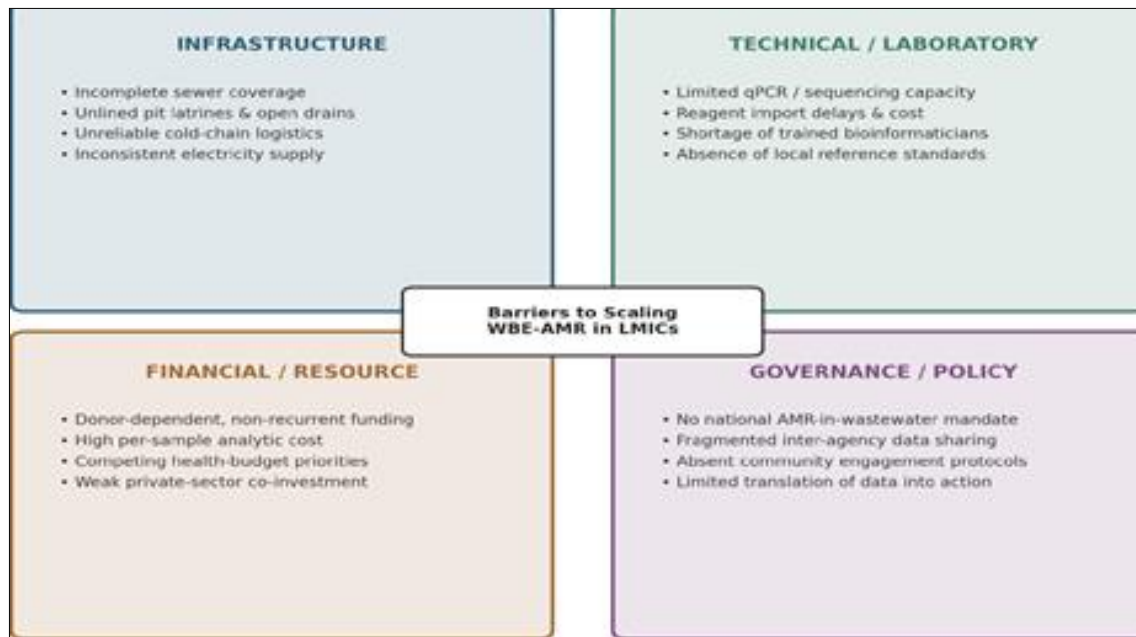


Fig 3: A four-domain framework of implementation barriers to wastewater-based AMR surveillance in resource-limited settings.

Infrastructural constraints begin with the most basic precondition for wastewater surveillance: a functioning sewer network. Large fractions of the population in many LMICs rely on unlined pit latrines, septic systems, or open drainage rather than piped sewerage, meaning that a substantial share of any given city's population is structurally invisible to conventional wastewater-treatment-plant-based sampling regardless of how sophisticated the downstream laboratory methods become (Amin *et al.*, 2024; Khan *et al.*, 2025) ^[12, 48]. Even where sewer coverage exists, cold-chain logistics for sample transport and unreliable electricity supply for autosamplers, freezers, and PCR equipment introduce additional points of failure that are comparatively rare concerns in high-income programme design (Wardi *et al.*, 2024) ^[92]. Adams *et al.* (2024) ^[4] documented that even the United States' National Wastewater Surveillance System, built with substantial federal investment, took several years to achieve near-national coverage, a sobering benchmark against which to weigh the infrastructural ambitions of LMIC programmes operating with a fraction of the comparable resources.

Technical and laboratory constraints compound the infrastructural picture. Limited access to qPCR instrumentation, next-generation sequencing capacity, and the specialized reagents these methods require, often imported at considerable cost and subject to customs delays, restricts which methods are realistically available to a given laboratory (Anand Kumar, 2023) ^[13]. Perhaps more limiting still is the shortage of trained bioinformaticians capable of processing and interpreting metagenomic data, a skill set that remains scarce even in many high-income public health laboratories and that is disproportionately concentrated in a small number of well-resourced academic centres globally (Arbefeville *et al.*, 2024) ^[14]. The absence of locally validated reference standards and quality-control materials further complicates efforts to benchmark LMIC-generated data against international comparators, a gap that the qPCR-

versus-metagenomics comparison literature has begun to address methodologically but has not yet resolved operationally for most LMIC laboratories (Knight *et al.*, 2024) ^[50].

Financial constraints are perhaps the most consistently cited barrier across the literature reviewed here. Wastewater AMR surveillance programmes in LMICs are disproportionately donor-dependent and structured around fixed-term research grants rather than recurrent domestic health budgets, a financing model that produces exactly the kind of small-scale, geographically isolated pilot projects Punch *et al.* (2025) ^[74] identified in their scoping review (Rony *et al.*, 2023) ^[76]. Per-sample analytic costs for high-throughput qPCR panels or metagenomic sequencing remain substantial relative to national health budgets in low-income settings, and AMR surveillance must compete directly with more visible and politically prioritized health needs for the same constrained fiscal envelope (Ajulo & Awosile, 2024) ^[6]. Private-sector co-investment, which has helped underwrite some wastewater surveillance infrastructure in high-income settings, remains largely absent from LMIC programme financing models to date.

Governance and policy constraints represent the final, and arguably most tractable, domain. Even where technical capacity and financing align, few LMICs have formally integrated wastewater-based AMR surveillance into their National Action Plans for AMR, meaning that even successful pilot data generation may have no institutionalized pathway into policy response (Delpy *et al.*, 2024) ^[36]. Inter-agency data sharing between environmental, veterinary, and clinical health authorities remains fragmented in most settings, undermining the One Health integration that the underlying science of wastewater surveillance is specifically well-suited to support (Kasse *et al.*, 2025) . Schneider *et al.* (2024) ^[47, 80] found, even within the comparatively well-resourced context of United States public health agencies, that training needs assessments

consistently identified gaps in staff capacity to translate wastewater surveillance data into actionable public health guidance,

a finding that plausibly understates the equivalent gap in LMIC public health agencies operating with substantially thinner institutional capacity.

Table 3: Domain-Specific Barriers to Scaling Wastewater-Based AMR Surveillance in Resource-Limited Settings

Domain	Illustrative barriers	Downstream consequence	Representative source(s)
Infrastructure	Incomplete sewer coverage; unreliable electricity; limited cold-chain transport	Large population segments structurally excluded from sampling frames	Amin <i>et al.</i> (2024); Khan <i>et al.</i> (2025) ^[12, 48]
Technical / laboratory	Limited qPCR and sequencing capacity; reagent import delays; scarce bioinformatics expertise	Restricted method choice; delayed turnaround; unresolved data backlogs	Anand Kumar (2023); Arbefeville <i>et al.</i> (2024) ^[14]
Financial	Donor-dependent, non-recurrent funding; high per-sample analytic cost	Small-scale, geographically isolated pilots rather than sustained systems	Rony <i>et al.</i> (2023); Punch <i>et al.</i> (2025) ^[74, 76]
Governance / policy	No formal wastewater-AMR mandate in National Action Plans; fragmented inter-agency data sharing	Surveillance data generated but not institutionally actioned	Delpy <i>et al.</i> (2024) Schneider <i>et al.</i> (2024) ^[36, 80]

Data Standardization, Normalization, and Harmonization Challenges

A recurring theme across the methodological literature is that wastewater-based AMR surveillance data remain difficult to compare across programmes, laboratories, and countries, undermining exactly the kind of aggregated, cross-national evidence base that would be most useful for informing global AMR policy. Normalization, the process of adjusting raw target concentrations to account for dilution, flow rate, and the fecal content of a given sample, remains methodologically contested even within well-resourced programmes; different laboratories variably normalize to flow-weighted volume, human-associated genetic markers such as crAssphage or HF183, or physicochemical proxies, and no consensus approach has yet emerged (Schenk *et al.*, 2024) ^[79]. This lack of standardization is not a purely academic concern: it directly limits the comparability of resistance gene abundance data reported from different LMIC pilot programmes, making it difficult to distinguish genuine differences in underlying AMR burden from artefacts of differing normalization choices.

The absence of a harmonized, prioritized list of AMR targets for wastewater surveillance compounds the normalization problem. Unlike SARS-CoV-2 wastewater surveillance, which converged relatively quickly around a small number of standardized viral targets, AMR wastewater surveillance must grapple with a combinatorial explosion of possible gene targets, resistance mechanisms, and host organisms, and different research groups have made different, often locally motivated, choices about which targets to prioritize (Klümper *et al.*, 2025) ^[49]. Regional coordination efforts within the European Union, most notably the EU-WISH and EU-JAMRAI joint actions working toward implementation of Article 17 of the revised Urban Wastewater Treatment Directive, illustrate what a coordinated approach to target prioritization might look like, but no equivalent coordinating mechanism yet exists for LMIC contexts, where the specific resistance genes of greatest local clinical relevance may in any case differ meaningfully from the European priority list (Conforti *et al.*, 2026) ^[31].

Cajas-Corrales *et al.* (2025) ^[26] argue explicitly for global standardization of both genomic methods and wastewater-based epidemiology protocols specifically to enable meaningful cross-country comparison, a case that carries

particular weight for LMIC integration into global surveillance architecture: without a shared minimum reporting standard, data generated through painstaking, often donor-funded LMIC pilot work risks remaining scientifically interesting but practically un-integrable into systems like GLASS, which depend on comparable, aggregable inputs across participating countries (World Health Organization, 2025) ^[100]. The National Academies of Sciences, Engineering, and Medicine (2024) ^[67] reached a similar conclusion in the context of United States wastewater surveillance more broadly, recommending the development of interoperable, nationally coordinated data reporting standards as a precondition for realizing the full public health value of wastewater surveillance investment, a recommendation whose logic extends naturally, and arguably more urgently, to the international coordination challenge facing LMIC wastewater AMR programmes.

Ethical, Legal, and Equity Considerations

Wastewater-based surveillance is frequently described as ethically simpler than clinical surveillance because it does not require individual informed consent, samples are anonymous by design, drawn from an aggregated community source rather than an identifiable person. This framing, while not wrong, understates the distinct ethical terrain that community-level environmental surveillance actually occupies. Arefin *et al.* (2025) ^[17] argue, in a widely discussed justice-centred framework for wastewater surveillance governance, that the relevant ethical unit of analysis shifts from individual privacy to group-level concerns: which communities are sampled, whose water infrastructure is monitored, and who benefits from, versus who is potentially harmed by, the resulting data. Drawing on political theorist Nancy Fraser's tripartite framework of justice, Arefin *et al.* (2025) ^[17] identify three recurring patterns of ethical concern that this review adapts specifically to the AMR wastewater context in Figure 4: maldistribution, in which testing infrastructure and the benefits of resulting data are unevenly available across catchments; misrecognition, in which surveilled communities are framed primarily as sources of epidemiological risk rather than as beneficiaries or partners in the surveillance enterprise; and exclusion, in which the communities being sampled have no meaningful role in decisions about how surveillance is designed, governed, or acted upon.

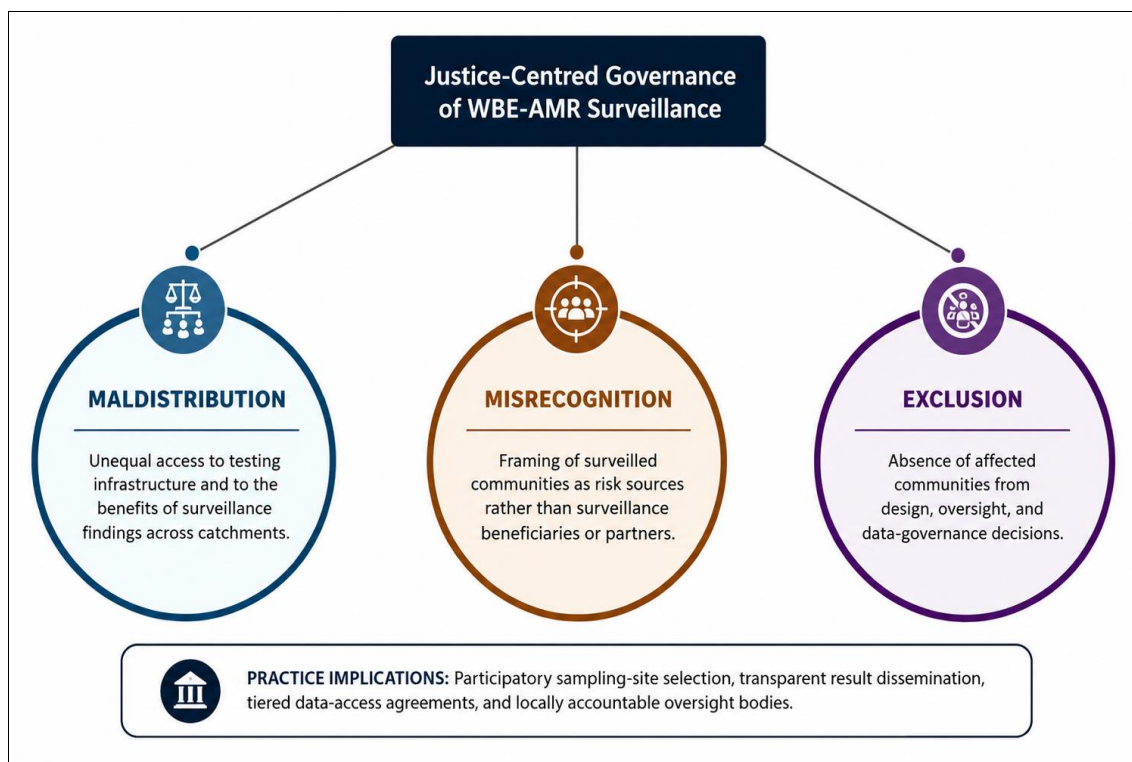


Fig 4: A justice-centred ethics framework applied to wastewater-based AMR surveillance.

These concerns are not abstract. Wittmer *et al.* (2025) ^[93] documented, through fieldwork across South Asian cities, how wastewater surveillance data is frequently produced, interpreted, and operationalized by external technical and donor actors in ways that leave the sampled communities themselves largely outside the decision-making process, a dynamic with clear resonance for LMIC AMR surveillance programmes that are, as noted above, disproportionately structured around externally funded pilot projects. The Association of State and Territorial Health Officials (2025) ^[18] developed a structured ethics framework specifically addressing privacy, stigma, and data stewardship concerns in wastewater surveillance, organized around the values of trust, reciprocity, justice, and the common good, a framework developed for a high-income policy context but whose underlying value structure translates reasonably well to LMIC settings, provided it is adapted rather than imported wholesale.

Stigma represents a particularly acute concern for AMR-specific wastewater surveillance, arguably more so than for pathogen surveillance during an acute outbreak. Because resistance gene abundance can plausibly be linked, at least in public perception, to antibiotic misuse, poor sanitation, or hospital infection control failures within a specific catchment, publicly reported data carries real potential to stigmatize the communities or institutions being sampled, a dynamic distinct from, and arguably more durable than, the stigma concerns raised during acute pandemic-era wastewater surveillance. Ethical governance frameworks developed for pandemic-era wastewater surveillance, useful as they are, were not designed with this specific dynamic centrally in mind, and the AMR wastewater surveillance

literature has only recently begun to grapple explicitly with how routine, ongoing environmental resistance surveillance should be communicated to and governed alongside the communities it draws from (Sousa-Carmo & Lincopan, 2025) ^[81].

Policy Integration: GLASS, One Health, and Regional Regulatory Frameworks

Table 4 summarizes several of the major policy and regulatory frameworks currently shaping, or poised to shape, wastewater-based AMR surveillance internationally, and their applicability to LMIC contexts varies considerably. GLASS remains the central global architecture for AMR surveillance data aggregation, but it was designed primarily around clinical and, more recently, tricycle-protocol One Health sampling rather than wastewater-based environmental surveillance specifically, meaning that a formal pathway for wastewater-derived AMR data to feed into GLASS reporting does not yet exist in most participating countries (World Health Organization, 2021, 2025) ^[98, 100]. The European Union's revised Urban Wastewater Treatment Directive, through its Article 17 provisions, represents the most advanced regional regulatory attempt to mandate AMR monitoring specifically within wastewater treatment infrastructure, but its applicability is explicitly limited to EU member states, and the infrastructure assumptions embedded in the directive, universal sewer connection, centralized treatment plants, reliable monitoring capacity, do not transfer straightforwardly to most LMIC contexts (Conforti *et al.*, 2026) ^[31].

Table 4: Selected Policy and Regulatory Frameworks Relevant to Wastewater-Based AMR Surveillance

Framework	Primary scope	Applicability to LMIC settings	Representative source(s)
WHO GLASS	Global aggregation of clinical (and tricycle One Health) AMR data	Participation mandate exists, but no formal wastewater-data reporting pathway yet established	World Health Organization (2021, 2025) ^[98, 100]
EU Urban Wastewater Treatment Directive, Article 17	Mandated AMR monitoring within EU wastewater treatment infrastructure	Limited; infrastructure assumptions (universal sewer connection) largely absent in most LMICs	Conforti <i>et al.</i> (2026) ^[31]
One Health National Action Plans	Cross-sectoral human–animal–environment AMR governance	Widely adopted in rhetoric; genuine data integration across sectors remains rare in practice	Delpy <i>et al.</i> (2024); Berendonk <i>et al.</i> (2015) ^[23, 36]
Justice-centred / community governance frameworks	Ethical oversight of community-level environmental surveillance	Adaptable in principle to LMIC contexts; requires local co-design rather than direct transplantation	Arefin <i>et al.</i> (2025) ^[17] ; Association of State and Territorial Health Officials (2025) ^[18]

The One Health framing that underlies much of the contemporary AMR policy discourse offers perhaps the most naturally LMIC-compatible entry point for wastewater surveillance integration, precisely because it does not require the kind of dedicated, AMR-specific regulatory infrastructure that the EU directive assumes. Delpy *et al.* (2024) ^[36], in a scoping review of integrated AMR surveillance systems, found that One Health framing has become nearly universal in policy rhetoric but that genuine cross-sectoral data integration, linking human, animal, and environmental surveillance streams into a single actionable system, remains rare in practice even in well-resourced settings, *let alone* LMICs. The environmental dimension of AMR specifically has historically been the weakest of the three One Health pillars in both research attention and policy integration, a gap that Berendonk *et al.* (2015) ^[23] identified as a priority concern over a decade ago and that Larsson *et al.* (2018) ^[53] subsequently reaffirmed remained largely unaddressed, underscoring how slowly environmental AMR surveillance, wastewater-based approaches very much included, has been absorbed into mainstream AMR policy architecture relative to clinical and agricultural surveillance streams.

Woolhouse *et al.* (2015) and Manaia (2017) ^[58, 94] both argue for risk-stratified rather than uniform approaches to environmental AMR monitoring, on the grounds that not every resistance gene detected in the environment carries equivalent public health significance, a point with direct relevance for LMIC policy design: given severe resource constraints, national AMR action plans in LMICs may be better served by prioritizing wastewater surveillance for a small number of clinically actionable, high-risk resistance mechanisms, carbapenemase genes, colistin resistance determinants such as *mcr-1*, and extended-spectrum beta-lactamases prominent among them, rather than attempting the comprehensive resistome characterization that better-resourced metagenomic programmes can pursue (Martinez *et al.*, 2015; Rebelo *et al.*, 2018; Wang *et al.*, 2018) ^[60, 75, 91].

Future Directions and Research Priorities

Several converging trends suggest plausible pathways toward more sustainable wastewater-based AMR surveillance in LMICs over the coming years, even as the barriers documented above remain substantial. First, the field-deployable long-read sequencing pilots described in Uganda (Khan *et al.*, 2025) ^[48] and the low-cost qPCR array approaches validated across multiple LMIC contexts (Al-Mustapha *et al.*, 2025; Talat *et al.*, 2025) ^[8, 85] suggest that per-sample technical costs are likely to continue declining,

potentially narrowing, though certainly not eliminating, the financial barrier documented in Table 3. Second, the repurposing of COVID-19-era wastewater surveillance infrastructure, cold-chain logistics, trained sampling personnel, established community relationships, for AMR applications represents a genuinely underexploited opportunity; the Ethiopian experience described by Abera Dinssa *et al.* (2024) ^[2] illustrates how existing pandemic surveillance capacity can plausibly be extended toward AMR monitoring at comparatively modest marginal cost, a model that merits deliberate replication rather than incidental discovery in other LMIC contexts where pandemic-era infrastructure remains underutilized.

Third, the accelerating integration of climate considerations into AMR research carries direct relevance for LMIC wastewater surveillance design specifically. Fernández Salgueiro *et al.* (2024) and Meinen *et al.* (2023) ^[40, 62] both document a strengthening evidence base linking rising temperatures and shifting precipitation patterns to increased antibiotic resistance gene abundance and transmission, a relationship of particular concern for LMICs situated in tropical and monsoon-affected regions where climate change impacts on water infrastructure are projected to be most severe. Barrantes-Jiménez *et al.* (2025) ^[20] demonstrated that seasonal and pollution-related variation in tropical urban river resistance gene abundance can be substantial, a finding with direct implications for how LMIC wastewater surveillance programmes should design sampling frequency and interpret temporal trends, given that the seasonal dynamics well-characterized in temperate European contexts (Schages *et al.*, 2020) ^[78] may not transfer directly to tropical and subtropical LMIC settings. Fourth, artificial intelligence and machine learning applications, while currently trained predominantly on high-income-country datasets, carry genuine potential to reduce the bioinformatics capacity barrier if development priorities shift explicitly toward LMIC-relevant training data and deployment contexts. Kasse *et al.* (2025) ^[47] and Bilal *et al.* (2025) ^[24] both argue that AI-assisted triage of environmental AMR signals could substantially reduce the specialized human capital required to interpret metagenomic data, provided the underlying models are validated on microbial community backgrounds genuinely representative of LMIC wastewater matrices rather than extrapolated from high-income reference datasets, a caution that should temper enthusiasm for immediate, unmodified transfer of existing AI tools into LMIC surveillance pipelines.

Finally, governance innovation may prove at least as consequential as technical innovation in determining

whether wastewater-based AMR surveillance achieves sustained LMIC integration. The justice-centred governance frameworks developed by Arefin *et al.* (2025) ^[17] and the structured ethics approaches advanced by the Association of State and Territorial Health Officials (2025) ^[18], while developed in and for high-income policy contexts, offer a starting template that LMIC public health authorities, research consortia, and international funding bodies could adapt collaboratively, provided that adaptation process itself involves meaningful participation from the communities and countries whose wastewater is being surveilled, rather than repeating the pattern of externally designed frameworks being applied to LMIC contexts without local input that this review has documented as a recurring structural weakness across the technical, financial, and governance dimensions of the field.

Conclusion

Wastewater-based epidemiology has matured, over the past several years, from a pandemic-era emergency tool into a genuinely versatile platform for population-level infectious disease and resistance surveillance. The evidence reviewed here suggests that the underlying biological premise transfers reasonably well to low- and middle-income settings: resistant organisms and resistance genes are detectable in LMIC wastewater using field-adapted, appropriately modest technical approaches, even where sewer coverage is partial, electricity supply is unreliable, and laboratory infrastructure is limited. What remains unresolved is not whether the method works in principle, but whether the surrounding ecosystem of financing, governance, data standardization, and community engagement can be built out with the same seriousness of purpose that has, to date, been directed primarily at the underlying laboratory science.

Four conclusions follow directly from this synthesis. First, methodological choices are not neutral: the trade-offs between culture-based, qPCR-based, and metagenomic approaches carry real consequences for what a given LMIC programme can realistically detect within its resource envelope, and programme design should be explicit about these trade-offs rather than defaulting uncritically to whichever method a funding partner happens to supply. Second, the barriers to scale-up are structurally interlinked rather than independent, meaning that addressing financing alone, without parallel attention to infrastructure, technical capacity, and governance mandates, is unlikely to produce durable surveillance systems. Third, data standardization is not a peripheral technical concern but a precondition for LMIC-generated surveillance data to be meaningfully integrated into global architectures such as GLASS, and international coordination on this front deserves far more attention than it has so far received. Fourth, the ethical terrain of wastewater-based AMR surveillance, centred on community-level rather than individual-level concerns around stigma, benefit-sharing, and governance voice, requires deliberate, locally grounded attention rather than the uncritical transplantation of frameworks developed elsewhere.

None of this diminishes the underlying promise of the approach. A surveillance method that can generate meaningful population-level resistance data without depending on the clinical infrastructure that LMICs so often lack represents a genuinely valuable complement to, rather

than a replacement for, conventional AMR surveillance. Realizing that promise at scale, however, will depend less on further proof-of-concept studies demonstrating technical feasibility, a case that has by now been made repeatedly and convincingly, and more on the harder, slower work of building sustainable financing models, harmonized data standards, and governance structures that treat the communities whose wastewater is being surveilled as partners in the endeavour rather than merely as its subjects.

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