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Inadequate waste management drives the spread of antimicrobial resistance across the watershed of a large African city

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ABSTRACT

Rapid urbanization in the Global South consistently outpaces sanitation infrastructure, transforming urban rivers into AMR reservoirs. Yet watershed-scale quantification of resistomes in such settings remains scarce, limiting exposure risk assessment and intervention planning. Here we present the most detailed spatial baseline for waterborne AMR in sub-Saharan Africa, using the Akaki River (Addis Ababa, Ethiopia) as a model system for rapidly urbanizing megacity watersheds with inadequate sanitation. We sampled 38 sites across an extreme pollution gradient during the dry season, when low flow produces maximum pollutant concentrations and the highest resident exposure risk. Water quality, microbiome marker data, 309 antibiotic resistance genes (ARGs), and 57 mobile genetic elements (MGEs) were quantified by high-throughput quantitative PCR. ARG and MGE abundances in central urban reaches exceeded 10^{10} gene copies L^{-1}, indistinguishable from untreated sewage. Critically, clinically important ARGs co-occurred widely with broad-host-range MGEs, including the IncP-1α plasmid backbone, IS*26*, IS*Ecp1*, IS*6100*, and class 1 integron integrase genes, indicating that ARGs are primed for potential horizontal gene transfer in waters where hundreds of thousands of Addis residents bathe, wash, and irrigate food crops. Dissolved oxygen depletion and elevated NH_4-N correlated significantly with resistome expansion ($p < 0.01$), serving as robust chemical proxies for diffuse, system-wide raw sewage inputs that carry the actual mechanistic AMR drivers (e.g., heavy metals, antibiotic residues, and resistant hosts). The 38-site spatial design and dry-season worst-case conditions together provide a replicable model for AMR exposure assessment in comparable Global South cities.

1. Introduction

The global spread of antimicrobial resistance (AMR) is recognized as one of the most consequential environmental health challenges of our era, with an estimated 1.27 million deaths attributable directly to AMR infections annually and projections of far greater numbers in the future (Murray et al., 2022). While clinical transmission pathways receive the greater attention, environmental reservoirs, particularly urban

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waterways in the Global South, represent critical but under-characterized vehicles of widespread AMR spread (Graham et al., 2019, Larsson and Flach, 2022). In rapidly urbanizing cities where sanitation infrastructure consistently lags population growth, rivers function not merely as receptacles for untreated domestic wastewater, agricultural runoff, and industrial effluents, but as active biological "reactors" where antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), and pharmaceutical residues comingle under conditions conducive to genetic exchange and resistome expansion (Gao et al., 2024, Mao et al., 2025).

The epidemiological significance of environmental AMR is not simply a question of how many ARGs are present in each waterway, but of whether those genes are structurally positioned for transfer to other microbes, especially pathogens. Mobile genetic elements, such as plasmids, transposons, integrons, and insertion sequences, are the principal vectors of horizontal gene transfer (HGT), enabling ARGs to move between organisms in the environment and clinically relevant species, including across taxonomic boundaries (Partridge et al., 2018, Razavi et al., 2020). Specifically, these MGE classes drive AMR transmission and selection through distinct mechanisms: broad-host-range conjugative plasmids enable inter-phyla transfer (Shintani et al., 2015); integrons (particularly class 1) capture and assemble multiple gene cassettes into multi-drug resistance arrays (Cambray et al., 2010); and insertion sequences frequently form composite transposons or provide strong promoters to overexpress downstream ARGs (Harmer and Hall, 2015, Varani et al., 2021). In polluted urban rivers, where hypoxic, nutrient-rich conditions simultaneously increase bacterial density, stress-induced competence, and conjugation frequency, the co-occurrence of high ARG loads with diverse MGEs creates conditions that can potentiate accelerated resistome evolution (Jong et al., 2020, Mao et al., 2025). Characterising this co-occurrence is, therefore, not a secondary concern but a primary indicator of environmental AMR hazard - the difference between a river that passively carries ARGs and one that actively selects them and promotes evolution and diversification.

Despite the urgency of this problem, comprehensive watershed-scale studies that simultaneously quantify resistome diversity, MGE abundance, and their co-occurrence structure in lower-income country (LIC) settings are remarkably scarce (Chen et al., 2024, Rony et al., 2023). Data from sub-Saharan Africa are especially limited (Abia et al., 2023, Sartorius et al., 2024), creating blind spots in global AMR surveillance. For example, the Akaki River in Addis Ababa, Ethiopia, represents a well-documented case of extreme urban water quality degradation, ranking among the world's most contaminated rivers (Wilkinson et al., 2022), with rapid urbanization transforming water quality from 'poor' to 'seriously polluted' between 1991 and 2021 (Gule et al., 2023).

Previous work on the Akaki has examined microbiome community structure (Hiruy et al., 2022), targeted ARG detection in sediments and water at selected sites (Yitayew et al., 2022), water quality and fecal contamination patterns (Haile et al., 2025), and WWTP performance (Haileselassie et al., 2025). However, these earlier approaches relied on limited spatial sampling or targeted narrow gene profiles, constraints that failed to resolve complex, watershed-scale ARG-MGE co-occurrence networks. This is a critical gap, i.e. without a comprehensive quantitative baseline across a wide range of sites and pollutant gradients, interventions cannot be rationally prioritized and progress cannot be measured.

The watershed features of the Akaki catchment are not unique to Addis Ababa. Local municipal WWTPs serve less than one-quarter of residential households and the sewage collection system is in poor repair and highly fragmented. Open defecation is also prevalent, livestock waste management is limited, and uncontrolled industrial discharges are common, conditions characteristic of dozens of rapidly growing cities across sub-Saharan Africa, South Asia, and Latin America (Graham et al., 2024, UNEP, 2023). Cities such as Kampala, Nairobi, Dhaka, Karachi, and Kathmandu share this sanitation deficit resulting from rapid urbanization, and where AMR data exist, they have similar expansive waterborne resistomes (Gao et al., 2024, Mittal et al., 2019, Yadav et al., 2021). Therefore, the Akaki watershed, our focus here, functions not merely as a case study of one African city but as an example of a globally prevalent problem and findings here have implications for hundreds of millions of urban residents around the world.

Here, a comprehensive watershed-scale assessment was performed across 38 locations in the Akaki River watershed. Sampling was conducted during the dry season, which represents worst-case conditions, i.e. minimum streamflow produces maximum pollutant concentrations, providing an upper-bound resistome conditions and exposures to residents. The breadth of the 38-site design, spanning agricultural upstream areas to a dense urban core to peri-urban downstream reaches, provides spatial resolution needed to characterize zone-specific resistome dynamics, identify possible AMR drivers, and assess co-occurrence genetic networks with statistical rigor. Specifically, here we: (1) establish a detailed quantitative spatial baseline for ARG and MGE diversity and abundance across the whole catchment; (2) characterize the co-occurrence of ARGs and different MGEs to assess potential gene mobilization and HGT risk; (3) identify the possible environmental drivers of resistome distribution; and (4) put findings into a global context to allow transferable policy guidance. Results provide both the essential data needed to prioritize infrastructure improvements in Addis Ababa and a framework for understanding the AMR problem in any resource-limited urban watershed.

2. Materials and methods

2.1. Study area and sample collection

The Akaki River watershed is in central Ethiopia and forms the headwater of the Awash River system. Covering over 1,500 km^2, the catchment drains diverse rural, urban, and peri-urban areas in and around Addis Ababa, the economic and political hub of the country. The two major tributaries of the Big Akaki River, the Kebena and the Bulbula, drain contrasting catchments. The Kebena primarily drains residential, commercial, and hospital catchment areas in the city, whereas the Bulbula drains cultivated and grazing lands and peri-urban settlements. Most major hospitals are situated along the Kebena corridor. The downstream Big Akaki reach receives the combined effluents of both tributaries, alongside additional inputs from local industrial sites, quarries, irrigated agriculture, and peri-urban residential spaces.

For spatial analysis, sampling sites were categorized into three zones (Fig. 1): Upstream (n = 9), primarily agricultural areas upstream of the city with minimal direct sewage inputs; Central (n = 26), largely urbanized areas within Addis Ababa with intense human activity and diverse waste discharges; and Downstream (n = 3), river water exiting the urban core and receiving suburban and agricultural runoff. Specifically, nested sampling at confluences (e.g., S24u and S31u) was utilized to differentiate upstream tributaries from immediate localized pollution, while the Downstream zone (n=3) deliberately targeted sites distant from direct industrial outfalls to capture aggregated watershed exit signals. To enhance the spatial clarity of the data presentation while maintaining the project's historical site designations, the suffixes 'u', 'c', and 'd' have been appended in the text to site numbers (e.g., S24u, S45c, S71d) throughout the manuscript to denote their zone.

Sampling was conducted in December 2023, during the dry season. This season was deliberately chosen for two methodological reasons. First, dry-season low-flow conditions produce maximum pollutant concentrations (Knapp et al., 2012), representing the upper bound of the AMR exposure risk faced by the approximately 600,000 residents who have direct contact with Akaki River water for washing, bathing, livestock watering, and crop irrigation (Haile et al., 2025, Wang et al., 2022). Second, the absence of wet-season dilution and variable stormwater flushing provides more stable, interpretable flow conditions, enabling more robust identification of chronic pollution sources and

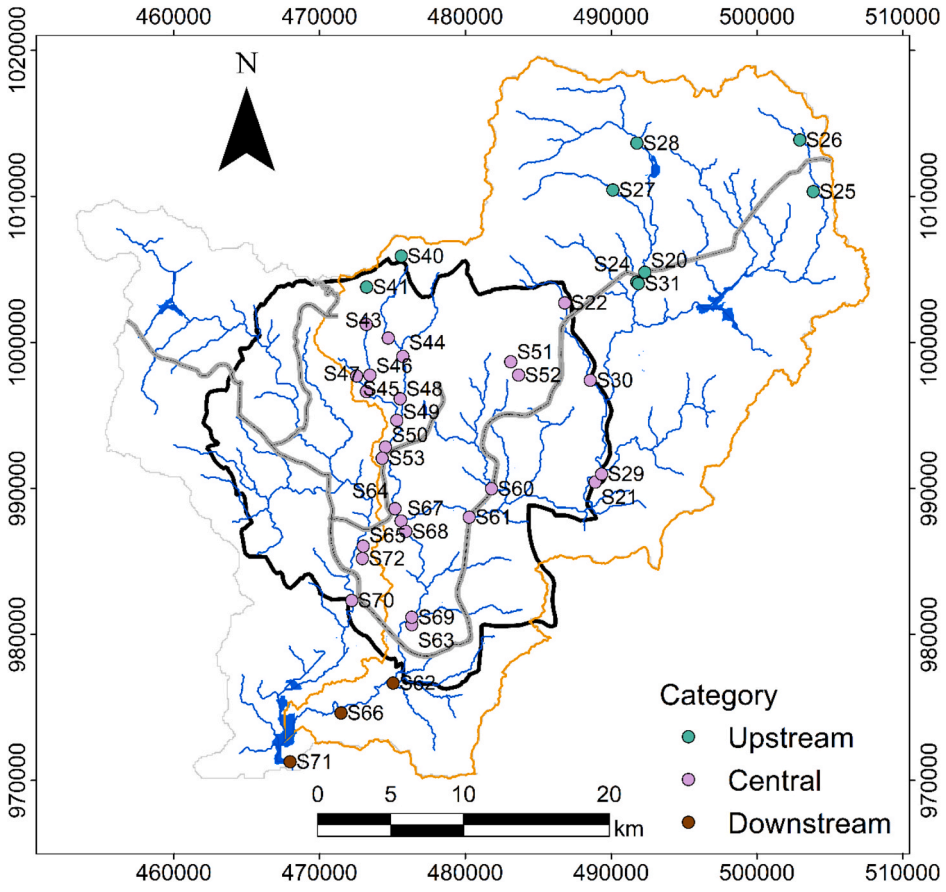


Fig. 1. Sampling sites in the Akaki River watershed, Ethiopia. The map displays the distribution of 38 sampling locations categorized into Upstream, Central, and Downstream zones along the river network based on geographic and anthropogenic features. To facilitate spatial identification in subsequent text and figures, site names are appended with suffixes corresponding to their respective zones: 'u' (Upstream), 'c' (Central), and 'd' (Downstream) (e.g., S45c).

ambient resistomes across the watershed. The spatial breadth of the 38-site design provides statistical compensation for the single time-point: the extreme within-campaign gradient (spanning agricultural headwaters to hypoxic urban reaches) capturing biological and environmental variations that fundamentally characterizes the system. Future seasonal sampling campaigns are planned and will build on this spatial baseline.

Water samples (1 L each) were collected and transported in an icebox to the laboratory. Each sample was filtered through 0.22 μm polycarbonate membrane filters, and filters were stored at −18°C until DNA extraction.

2.2. DNA extraction and high-throughput quantitative PCR (HT-qPCR)

Total DNA was extracted from membrane filters using the FastDNA™ SPIN Kit for Soil (MP, USA) following manufacturer protocols. DNA quality and quantity were assessed using a Qubit 4 Fluorometer (Thermo Fisher Scientific). High-throughput quantitative PCR (HT-qPCR) was performed using a SmartChip Real-Time PCR system (Takara Bio) targeting 309 ARGs (covering all major drug classes), 57 MGEs (including transposases, integrases, and plasmid-associated genes), ten metal resistance genes, seven taxon-specific marker genes, and the bacterial 16S rRNA gene as an indicator of total bacterial abundance (the complete list of targeted genes and primers are provided in Supplementary Table 1) (Li et al., 2025, Li et al., 2024).

Each reaction was 100 nL with three technical replicates per assay. Genes detected in at least two replicates with a cycle threshold Ct $\leq$ 31 were considered positive. Gene copy number was estimated as 10^((31−Ct)/(10/3)) (Looft et al., 2012). Relative abundance was calculated by dividing each gene copy number by the 16S rRNA gene copy number in the same sample. Absolute 16S rRNA gene copy numbers were calculated from standard curves and normalized to copies per litre of water. Standard curves were generated using ten-fold serial dilutions of a standard plasmid (e.g., containing the targeted 16S rRNA gene fragment) ranging from 10^4 to 10^8 copies/μL. The amplification efficiency was 95% and the coefficient of determination of the regression line (R^2) was consistently greater than 0.99.

2.3. Environmental and water quality parameters

Temperature, altitude, total dissolved solids (TDS), pH, dissolved oxygen (DO), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and phosphate (PO_4-P) were quantified using standard methods concurrent with microbial sampling (Haile et al., 2025).

2.4. Statistical analysis

Alpha-diversity indices (Richness, Shannon, Simpson) for ARGs and MGEs were calculated using the vegan package in R (Oksanen et al., 2013). Group differences were tested with Wilcoxon rank-sum or pairwise t-tests based on normality (Shapiro-Wilk). Beta-diversity was assessed by principal coordinate analysis (PCoA) using Bray–Curtis dissimilarity; PERMANOVA (adonis) tested group differences. Random Forest analysis identified key genes driving group separation (randomForest package). Redundancy analysis (RDA) and correlation analysis explored ARG/MGE–environment relationships; RDA significance was tested by permutation-based ANOVA (999 permutations). Co-occurrence between ARGs and MGEs was characterized using Spearman correlation with Mantel testing and envfit analysis.

Visualization used ggplot2 and ComplexHeatmap (Gu and Hübschmann, 2022, Wickham, 2011).

3. Results

3.1. Bacterial abundance and diversity of antibiotic resistance genes

Absolute abundances of bacterial 16S rRNA genes showed clear spatial patterns across the Akaki watershed (Fig. 2A, B). The Central zone exhibited the highest bacterial levels, with median values frequently exceeding 10^{10} copies L^{-1}, significantly higher than most Upstream sites (Kruskal-Wallis test, $p < 0.05$). Downstream zone levels were comparable to the Central zone, although sample numbers were limited (n = 3).

The same pattern was apparent for AMR markers. ARG richness (number of detected ARG subtypes) was highest in Central zone followed by Downstream sites, with Upstream sites consistently lower (Fig. 2C). Of the 309 ARGs and 57 MGEs targeted by HT-qPCR, only 22 ARGs and four MGEs were not found anywhere in the watershed, a strikingly comprehensive detection rate reflecting the severity of AMR contamination. The number of unique ARGs detected per sample ranged from 41 to 161 (median 92) Upstream, 90 to 250 (median 186) at Central sites, and 133 to 166 (median 159) Downstream. Peak ARG counts were recorded at S45c (Kebena River, Tewodros Square), S48c (Kebena River, near Urael Church), and S72c (influent of the Akaki WWTP).

The most abundant ARGs varied by zone, revealing zone-specific resistance signals (Fig. 3). At Upstream sites, *tetB(P)* (mean relative abundance 0.03 copies/16S copies) was consistently dominant, followed by *qacF_H* (0.019) and *qacH_351* (0.016). In the Central zone, *qacH_351* (0.049) and *qacF_H* (0.049) were substantially elevated alongside *tetB (P)* (0.042) and *sul1* (0.040). Downstream, *sul1* (0.114) was predominant, reflecting the cumulative merging of urban wastewater with agricultural runoff, while *qacH_351* (0.059) and *qacF_H* (0.038) remained elevated. MGEs showed corresponding zone-specific patterns: *intI1_337* and IS*6100* were most abundant upstream; both, together with *tnpA-1*, *tnpA-2*, *ISAba3-Acineto*, and *ISPps1-pseud*, reached peak abundances in the Central zone; and *IS6100*, *intI1_337*, and *tnpA-1/2* remained highly elevated downstream (Fig. 3).

3.2. Spatial distribution and community structure of ARGs and MGEs

Alpha-diversity indices for ARGs and MGEs differed significantly across zones (Fig. 4A-D). For ARGs, richness was significantly higher in the Central than the Upstream zone ($p < 0.001$), while the Simpson diversity index differed significantly between the Central and Downstream zones ($p < 0.05$). For MGEs, richness significantly differed between the Upstream and Central zones ($p < 0.05$), and the Simpson index differed significantly between the Central and Downstream zones ($p < 0.05$).

At the drug class level, beta-lactam, multidrug, and aminoglycoside resistance genes were significantly elevated in the Central zone, with the Downstream zone also showing high abundances for these classes (see Fig. 4G). Tetracycline, MLSB, and rifamycin resistance genes were enriched in the Central zone but were lower Downstream. Sulfonamide resistance genes showed the opposite pattern, being more abundant Downstream, possibly reflecting the additive effect of agricultural AMR drivers on upstream urban wastewater.

Among genes with comparatively high Upstream relative abundance, *qacH_351*, *qacF_H*, *sul1*, and *tetB(P)* deserve attention: their upstream presence indicating background anthropogenic pressure exists even at less-impacted sites. MGE abundance patterns are like the ARG patterns, with the Central zone showing highest MGE levels (Fig. 4E). Among metal resistance genes, *merA* and *copA* were most prevalent across

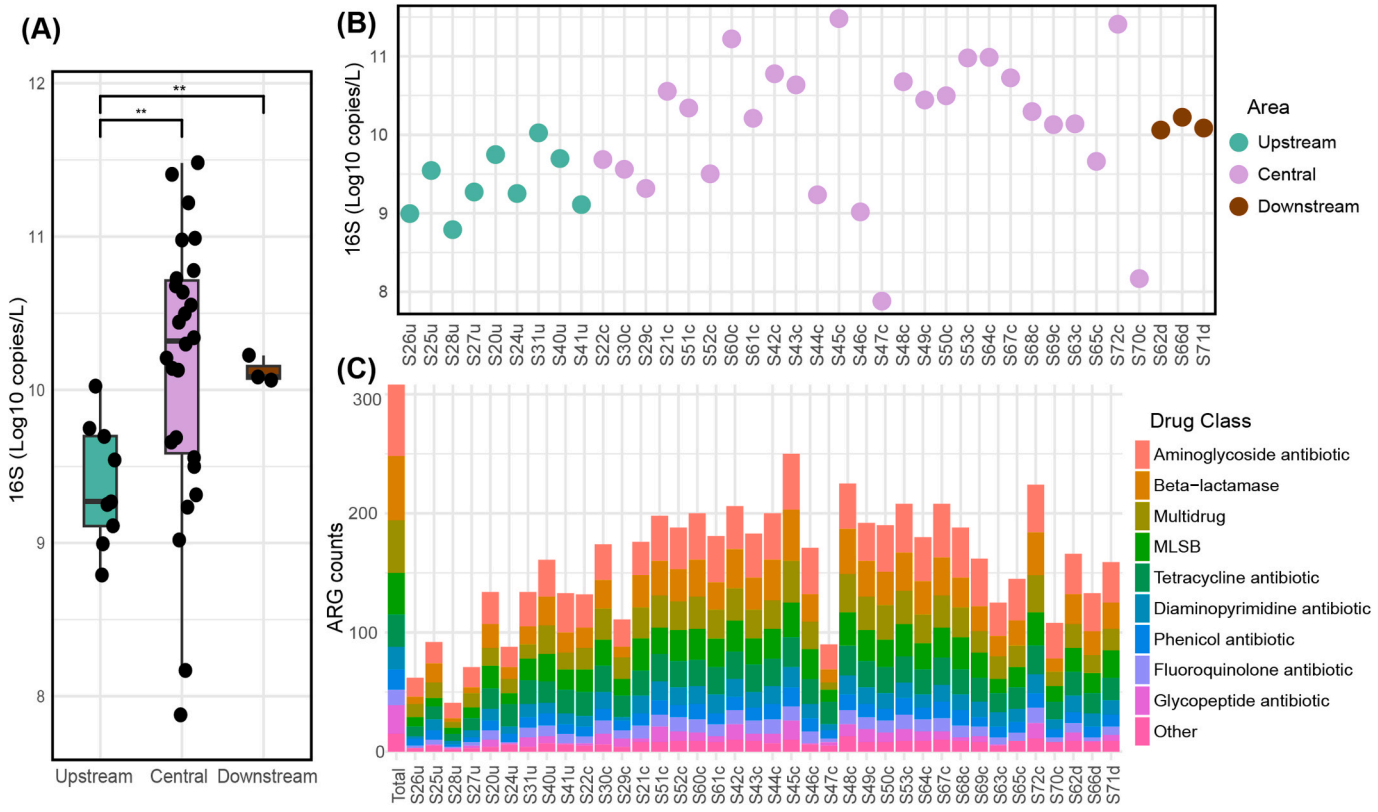


Fig. 2. Bacterial abundance and antibiotic resistance gene (ARG) richness across the Akaki River watershed. (A) Boxplot summarizing the distribution of 16S rRNA gene abundance in Upstream (n = 9), Central (n = 26), and Downstream (n = 3) regions. (B) Absolute abundance of the bacterial 16S rRNA gene at individual sampling sites. (C) ARG richness at the drug class level across sampling sites. Abbreviations: ARG, antibiotic resistance gene; rRNA, ribosomal RNA.

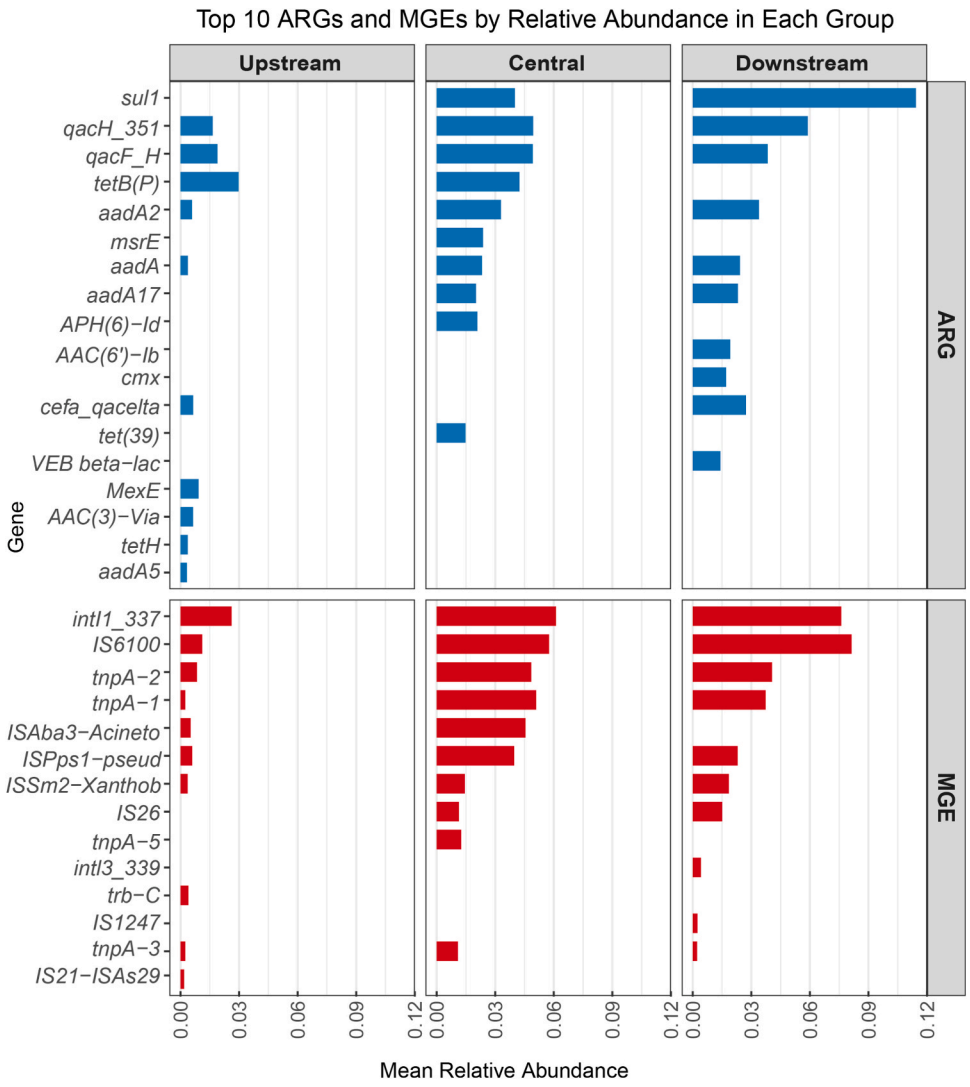


Fig. 3. Top high-abundance ARGs and MGEs across distinct sampling zones. Bar charts display the top 10 ARGs (upper panels) and MGEs (lower panels) with the highest mean relative abundance within each respective group (Upstream, Central, and Downstream). Because the top-ranked genes vary by zone, a total of 18 unique ARGs and 14 unique MGEs are displayed across the panels.

individual sites (Supplementary SFig. 1B); Notably, the prominent outlier observed in the Upstream zone of the regional MRG boxplot (Fig. 4F) corresponds specifically to the exceptionally high *merA* abundance at site S40u (Supplementary SFig. 1B), suggesting a possible mercury contamination point source in the upper catchment.

Beta-diversity analysis (PCoA) revealed distinct clustering of ARG and MGE profiles by geographic zone (Fig. 5A-B). Central zone samples formed a coherent cluster clearly separated from Upstream sites. Random Forest analysis identified key genes driving this spatial differentiation (Fig. 5C-D): for ARGs, *arr-2*, *AAC(6')-Ib*, *APH(3')-Ia*, and bla_{CARB}; for MGEs, *trb-C*, *tnpA-1*, and *pBS228-IncP-1α*. Heatmaps (Supplementary SFig. 2-3) further illustrated the relative abundance of a curated set of 46 ARGs and 36 MGEs revealing distinct presence of ARGs like bla_{CARB}, *arr-2*, *APH(6')-Id*, *APH(3')-Ib*, and *ermB*, and MGEs like *tnpA*, *pBS228-IncP-1α*, *Tp614*, *IS630*, and *IS6100*. The identification of *pBS228-IncP-1α* is particularly notable. IncP-1α plasmids are the archetypal broad-host-range conjugative plasmid backbone, capable of replication and transfer across distantly related bacterial phyla, and their enrichment in the Central zone suggests a locus of potential AMR transmission and evolution.

3.3. Environmental drivers of ARG and MGE distribution

Correlation analysis and redundancy analysis (RDA) together identified the primary environmental determinants of resistome structure (Fig. 6). DO was significantly negatively correlated with ARG and MGE abundances across the catchment (Fig. 6A, D): as dissolved oxygen, a known indicator of organic pollution and decomposition, declined, ARGs and MGEs accumulated correspondingly. RDA identified DO and altitude (a proxy for urban-to-rural gradient) as significant influencers of both ARG and MGE community composition (Fig. 6B, E). The RDA model for ARGs was statistically significant (permutation-based ANOVA, $p < 0.05$). Environmental fitting (envfit) analysis (Fig. 6C) further confirmed that NH_4-N levels significantly correlated with ARG composition ($p < 0.01$), reinforcing that NH_4-N, a product of microbial decomposition, is a reliable marker of the conditions where elevated ARG loads exist. Although the RDA for MGEs was less statistically robust ($p > 0.05$), envfit confirmed DO ($p < 0.001$) and altitude ($p < 0.05$) as significant predictors of MGE distribution patterns (Fig. 6E, F).

Together, these spatial patterns suggest that the Akaki watershed resistome may be influenced more heavily by massive and cumulative, catchment-wide diffuse pollution rather than specific identifiable point sources, such as individual hospitals or industrial outfalls. The fact that

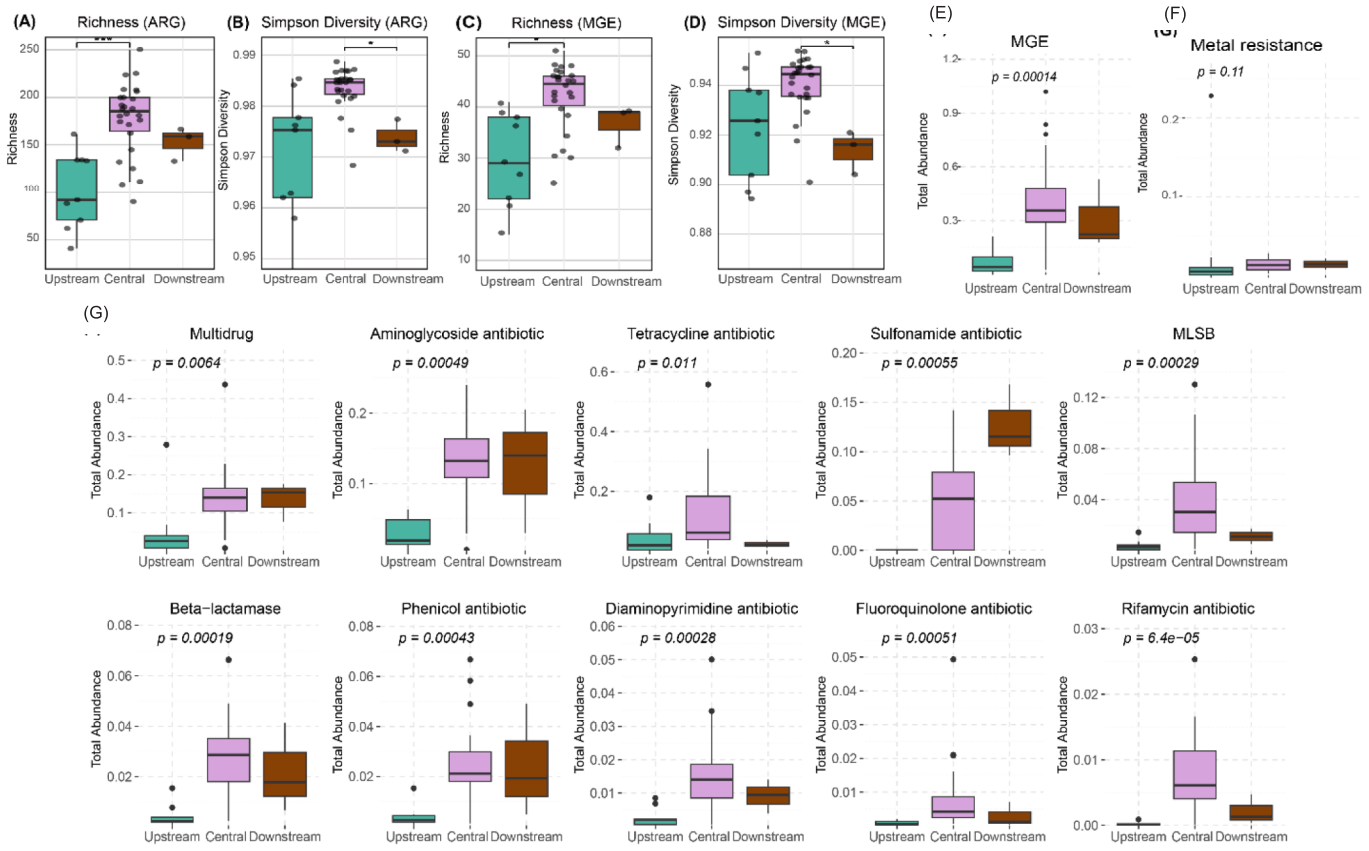


Fig. 4. Alpha-diversity and relative abundance of genetic elements across sampling zones. (A-B) Richness, and Simpson diversity of ARGs. (C-D) Richness, and Simpson diversity of MGEs. (E) Relative abundance of MGEs. (F) Relative abundance of metal resistance genes. (G) Relative abundance of ARGs grouped by drug class.

standard water quality parameters (e.g., DO, NH_4-N) act as robust chemical proxies for resistome conditions has practical implications. These parameters are cheap, simple to measure, and often included in routine water quality monitoring plans, suggesting they can serve as accessible indicators for waterborne AMR exposures, especially in places where metagenomic surveillance is not yet feasible.

3.4. Microbial correlates and ARG-MGE co-occurrence networks

Correlation and multivariate analyses revealed significant positive associations between taxon-specific marker genes, ARG drug classes, and MGEs (Fig. 7A, D; most $p < 0.01$). While the overall RDA model linking taxon-specific markers to ARG drug class levels was not significant ($p > 0.05$), *Firmicutes* emerged as an important contributor (Fig. 7B). In contrast, the RDA model relating taxon-specific markers to MGE distribution was highly significant ($p < 0.01$), with *Firmicutes* and *Enterococcus*-specific markers as key correlators (Fig. 7E). Envfit analysis further refined these associations: *Firmicutes* significantly explained variation in ARG profiles ($p < 0.001$), while significant explanatory taxa for MGE distribution included *Firmicutes*, *Enterococcus*, and *Acinetobacter baumannii* (Fig. 7C, F). The association of *A. baumannii* with MGE patterns is particularly significant given this organism's clinical importance as a multidrug-resistant nosocomial pathogen and its capacity to transmit and acquire resistance genes via multiple horizontal transfer mechanisms (Cho et al., 2020).

The ARG-MGE correlation and multivariate analyses (Fig. 8) revealed strong, statistically significant associations between specific MGE families and ARG drug classes. The correlation heatmap identified integrase- and transposase-associated elements, particularly *intI1_337* and IS*6100*, as explaining substantial variation in ARG drug class profiles ($R^2 > 0.36$, $p < 0.001$) (Fig. 8A). The corresponding RDA was highly significant (p = 0.001), revealing strong positive correlations between specific MGEs and multiple ARG classes simultaneously (Fig. 8B, C). Gene-level analysis using Random Forest-identified and high-abundance ARGs confirmed multiple strong co-occurrence relationships between MGEs (*tnpA*, *IncN_rep*, IS*1133*, IS*6100*, *intI1_337*) and ARGs (Supplementary SFig. 4).

4. Discussion

4.1. Characterization of one of the world's most AMR-impacted urban rivers

This study provides among the most detailed quantitative spatial descriptions of waterborne AMR in a sub-Saharan African city to date, and one of the most comprehensive in the Global South. Where previous studies on the Akaki described microbiome traits at selected sites (Hiruy et al., 2022), quantified specific ARGs in sediment and water samples (Yitayew et al., 2022), and-or assessed WWTP performance (Haileselassie et al., 2025), new work here provides a catchment-scale snapshot of the full magnitude and spatial distribution of waterborne AMR exposures to residents of Addis Ababa (Supplementary Table 2 and 3 for ARG, MGE, and pollution levels of all sites). The scale of associated environmental hazards and exposures is severe.

Absolute ARG and MGE abundances in the Central zone of the Akaki are indistinguishable from raw, untreated sewage (An et al., 2018, Rocha et al., 2019, Sowah et al., 2022), placing urban reaches in Addis Ababa among the most AMR-impacted waterways yet documented in

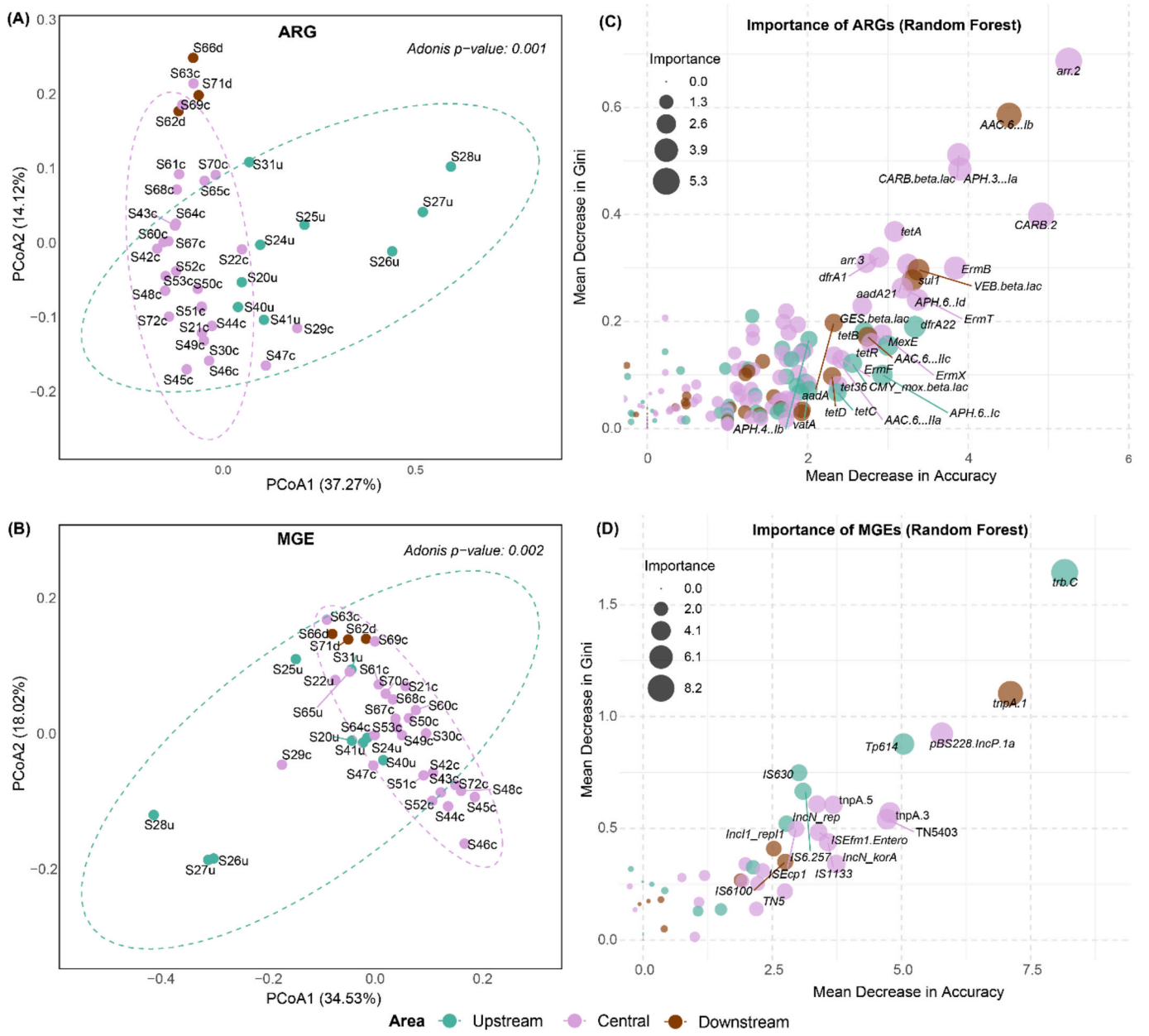


Fig. 5. Beta-diversity and key contributors to spatial differentiation of resistomes and mobilomes. (A) PCoA of ARG beta-diversity. (B) PCoA of MGE beta-diversity. (C) Random Forest analysis identifying key ARG contributors to spatial differences. (D) Random Forest analysis of key MGE contributors.

the world (Srathongneam et al., 2024, Zhou et al., 2024). However, this is not simply a water quality problem in an abstract sense: approximately 600,000 residents are estimated to have routine direct contact with Akaki River water, for washing, bathing, livestock watering, and irrigation of food crops consumed locally (Haile et al., 2025, Wang et al., 2022). While localized clinical reports on specific waterborne infections in this catchment remain scarce due to limited surveillance infrastructure, sub-Saharan Africa bears the highest age-standardized mortality rate associated with AMR globally (Murray et al., 2022). Thus, widespread environmental AMR exposures documented here represent a consequential public health threat, likely driving "silent" community-acquired infections rather than indicating a true low incidence of disease.

However, there have been recent efforts to improve the situation (Graham et al., 2024). Within this context, our new dry-season baseline provides a reference point against which future changes can be evaluated, such as planned increases in infrastructure investment for upgrading of existing WWTPs and construction of new facilities. It is important to note that while our results capture peak concentrations, they should not be generalized as a static, year-round baseline, as wet-season dilution will likely alter these exposure dynamics. As noted in the methods, future wet-season sampling campaigns are planned to establish a comprehensive year-round dynamic. Nonetheless, establishing an estimation of "worst case" conditions is not just a descriptive exercise but an essential precondition for future evidence-based environmental AMR governance in Addis Ababa and comparable cities.

4.2. *Mobilization potential: why co-occurrence of ARGs and MGEs matters for human and animal health*

A central finding of this study, and one with the most direct implications for human and animal health risk, is not simply that AMR genes are abundant in the Akaki - prior work suggested this (e.g., Yitayew et al., 2022). It is that those ARGs co-occur pervasively with a diverse

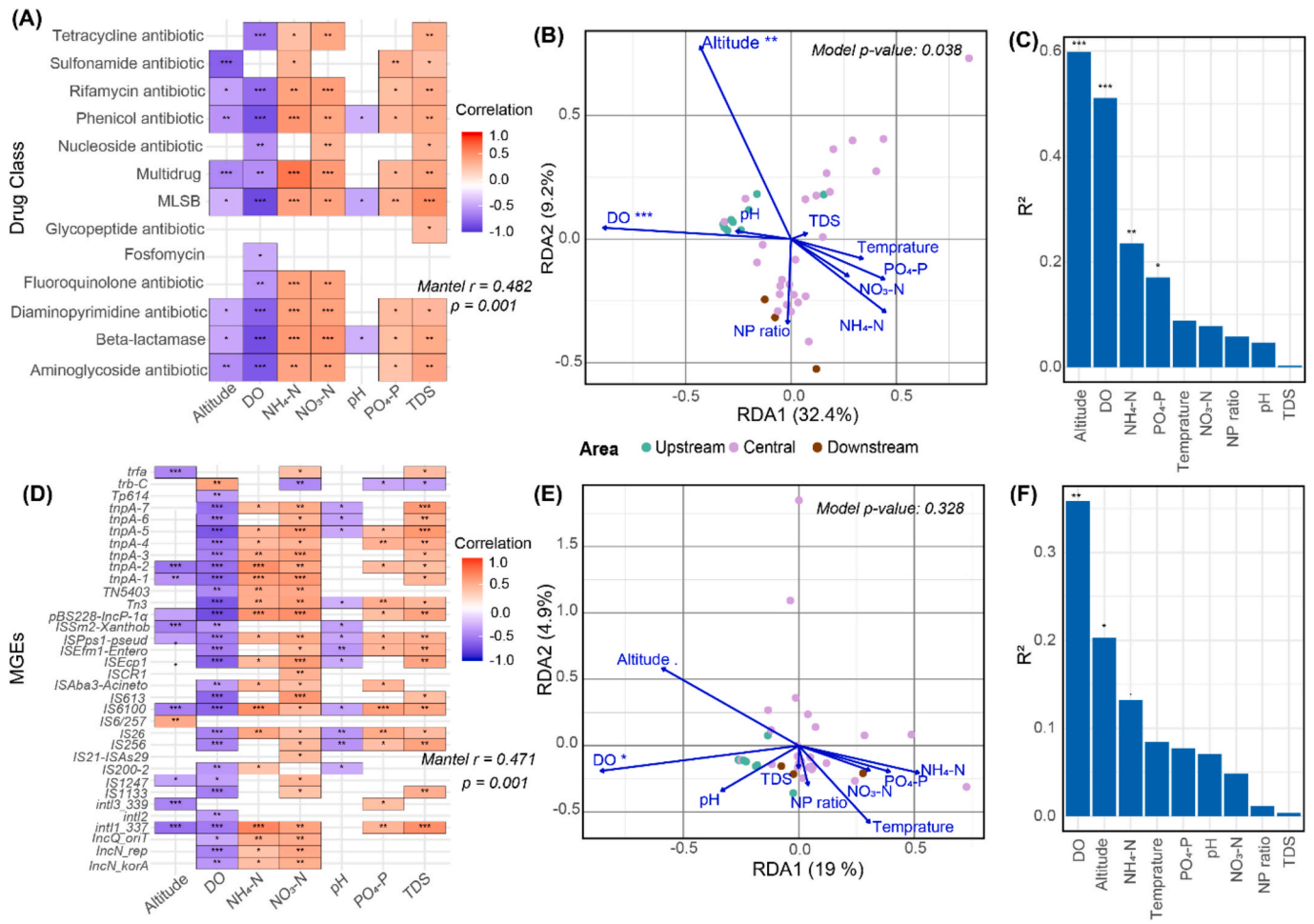


Fig. 6. Relationships between environmental parameters and the distribution of ARGs and MGEs. (A) Correlation heatmap with Mantel test results ($R^2 > 0, p < 0.05$) between environmental parameters and ARG drug classes. (B) Redundancy analysis (RDA) of environmental parameters and ARG drug classes. (C) Environmental fitting (envfit) analysis. (D-F) Equivalent analyses for MGEs.

array of MGEs that are specifically associated with inter-species and inter-phyla HGT. This distinction is critical: a river carrying large numbers of ARGs in stable, non-transferable chromosomal locations poses a very different public health risk from one where those same genes are physically associated with MGEs capable of transferring genes into new diverse bacterial hosts, including pathogens, under conditions present at the time of contact.

The MGE co-occurrence data presented strongly support the latter scenario. Six specific mobile elements deserve particular attention for their clinical significance (Table 1):

pBS228-IncP-1α plasmid backbone. The detection of pBS228-IncP-1α across Central and Downstream sites, and its identification as a primary discriminator of the Central Akaki resistome, is probably the most clinically relevant finding in this dataset. IncP-1α plasmids are the archetypal broad-host-range conjugative plasmid backbone, capable of autonomous replication and conjugative transfer across distantly related bacterial genera and phyla (Shintani et al., 2015, Thomas and Nielsen, 2005). Unlike narrow-host-range plasmids, IncP-1 plasmids can establish themselves in almost any Gram-negative organism, including environmental bacteria, animal-associated organisms, and clinically important pathogens, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*. Their co-occurrence with clinically important ARGs in waters in direct contact with residents means that every "human contact event" with river water represents a potential exposure to organisms carrying plasmids structurally capable of transferring those ARGs to bacteria already colonizing the human gut or respiratory tract. The *trb-C* gene, a conjugative transfer gene, further supports the potential for active conjugative transfer activity associated with these plasmids.

IS*26*. IS*26* is one of the most clinically important insertion sequences in global AMR epidemiology, documented as a primary driver of bla_{NDM}, bla_{KPC}, bla_{OXA}, and other carbapenem resistance gene mobilization in hospital-acquired pathogens worldwide (Harmer and Hall, 2015). Its detection at 29 of 38 sites, peaking at S45c on the Kebena River corridor (the hospital-proximate reach), and its co-occurrence with beta-lactam ARGs, represents a concrete mechanism by which carbapenem resistance, currently a last-resort treatment for many hospital-acquired infections, might be transferred from environmental strains to nosocomial pathogens. The spatial enrichment of IS*26* in the Central zone, where resident exposure is highest, amplifies this concern.

IS*Ecp1*. *ISEcp1* is a transposable element with a well-documented role in the mobilization of extended-spectrum beta-lactamase (ESBL) genes, particularly bla_{CTX-M} variants (Lartigue et al., 2006, Poirel et al., 2003). Its detection at 37 of 38 sites, including all upstream sites, indicates that ESBL mobilization is essentially ubiquitous across the entire watershed. The near-universal distribution of IS*Ecp1*, combined with the concurrent detection of extended-spectrum beta-lactamase genes (such as VEB-type variants) at 31 sites, suggests conditions amenable to ESBL gene acquisition prevail across the full spatial extent of the study area.

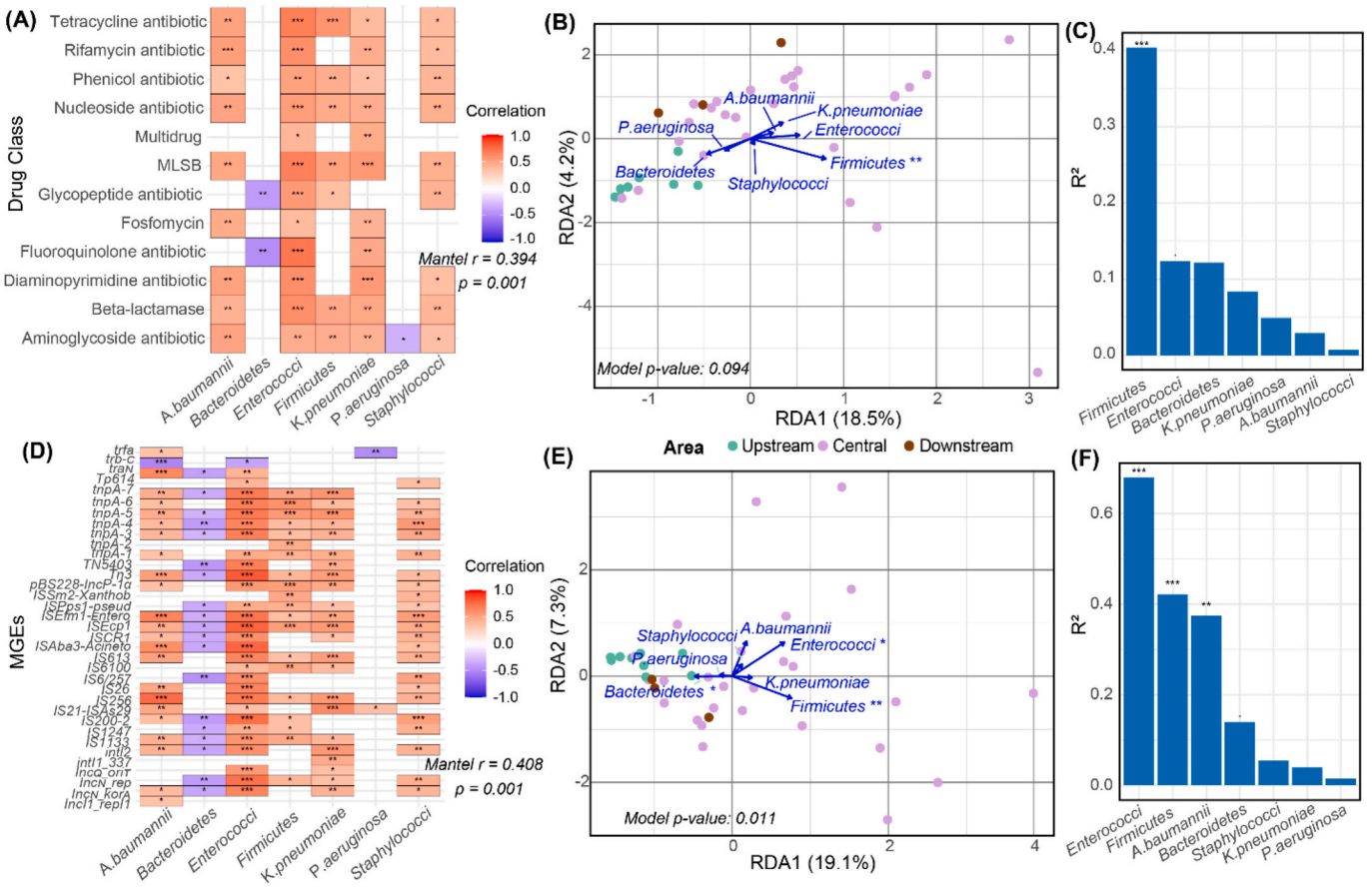


Fig. 7. Associations between potential microbial hosts (taxon-specific marker genes) and genetic elements. (A) Correlation heatmap with Mantel test results between taxon-specific marker genes and ARG drug classes. (B) RDA of taxon-specific marker genes and ARG drug classes. (C) Envfit analysis. (D–F) Equivalent analyses for MGEs.

***intI1_337* (class 1 integron integrase).** Class 1 integron integrase (*intI1*) is recognised as a proxy for anthropogenic impact in environmental samples and a frequent facilitator of resistance gene cassette assembly, enabling bacteria to acquire and express multiple resistance genes simultaneously (Cambray et al., 2010, Gillings et al., 2015). The detection of *intI1_337* at all 38 sites with the highest mean relative abundance of any MGE (0.054), and its identification as the primary driver of ARG drug class variation ($R^2 > 0.36$, $p < 0.001$), establishes that integron-mediated cassette assembly exists at a catchment scale. The co-occurrence of *intI1* with *sul1*, *dfrA*, *aadA*, and *cmlA* genes, all common integron cassette components, confirms that functional resistance cassettes are being maintained and potentially assembled in situ, not transported passively.

***ISAba3*.** *ISAba3*, an insertion sequence primarily associated with *Acinetobacter*, also warrants note. Clinically, *ISAba3* is infamous for its role in driving the overexpression of OXA-type carbapenemase genes, particularly $bla_{\text{OXA-58}}$, by providing strong upstream promoters (Chen et al., 2010). Its detection at 30 sites and peak abundance at S46c, combined with the significant correlation between *A. baumannii* marker genes and MGEs in envfit analysis, indicates that this important nosocomial pathogen is present and available for resistance mobilization across the watershed. *A. baumannii* is naturally transformable, capable of acquiring DNA from the environment, and has demonstrated the ability to survive outside clinical settings (Jawad et al., 1998). Its significant correlation with MGEs, alongside *Enterococcus* and *Firmicutes*, implies a "clinical-environmental-clinical" AMR cycle. The persistence of these potential hosts in river water, co-occurring with broad-host-range plasmids, creates high-risk exposure routes upon human contact, facilitating the re-entry of environmentally-adapted pathogens into healthcare settings.

***IS6100*.** The ubiquitous detection of IS*6100* across all 38 sampling sites, peaking alongside *intI1_337* in the Central and Downstream zones, represents another indicator of elevated AMR mobilization potential. Belonging to the same clinically important IS6 family as IS*26*, *IS6100* is highly active in forming composite transposons that capture and mobilize diverse ARGs (Varani et al., 2021). Furthermore, both IS*6100* and IS*26* frequently colocalize with class 1 integrons on broad-host-range plasmids, often flanking or truncating integrase genes to assemble multiple resistance cassettes, including those for aminoglycosides and tetracyclines (Tauch et al., 2002). Previous metagenomic studies in highly polluted agricultural environments have shown that IS*6100* abundance strongly correlates with the enrichment of multi-drug resistance clusters (Johnson et al., 2016). In the context of the Akaki River's severe pollution, the pervasive presence of IS*6100* suggests it might be an important molecular vehicle that allows microbial communities to adapt to mixed-waste environmental stressors, co-selecting for ARGs in the absence of antibiotic pressure.

Taken together, these MGE patterns show that the Akaki River is not simply a vehicle for ARG transport. It is a physicochemical and molecular mosaic for ARG mobilization, including via conjugative plasmids, transposases, integron integrases, and insertion sequences. The exposure risk this represents goes far beyond risk that would be implied by ARGs alone.

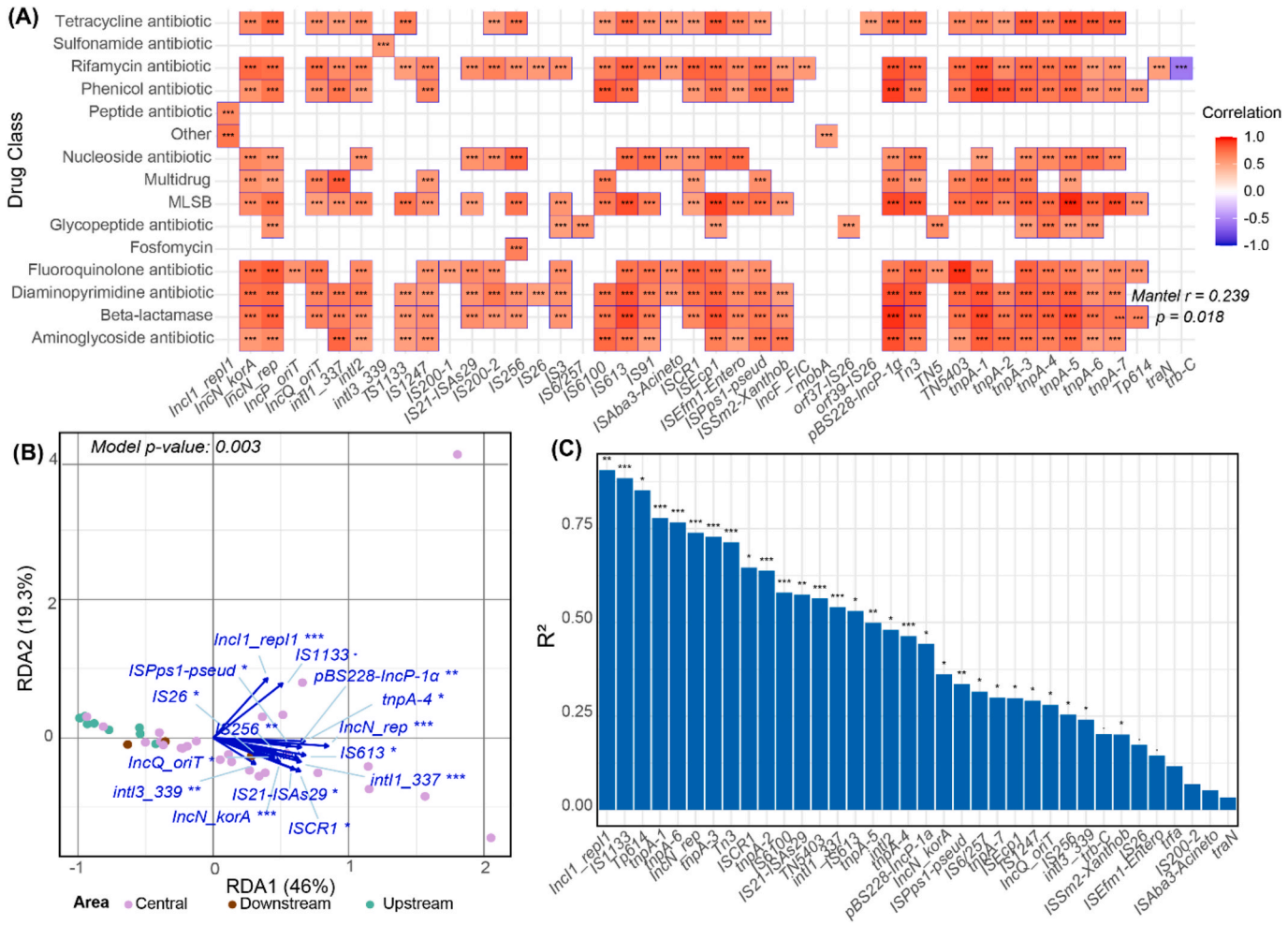


Fig. 8. Co-occurrence patterns and statistical associations between ARGs and MGEs. (A) Correlation heatmap ($R^2 > 0.36$, $p < 0.001$) evaluating relationships between specific MGEs and ARG drug classes. (B) RDA of ARG drug classes and MGEs. (C) Envfit analysis.

Table 1
Summary of key mobile genetic elements.

MGE	Detection Frequency	Peak Site	Clinical Relevance
pBS228-IncP-1α	35 of 38 sites	S21c	Archetypal broad-host-range conjugative plasmid; transfers ARGs across distantly related bacterial phyla.
IS*26*	29 of 38 sites	S45c	Primary driver of carbapenem resistance mobilization in hospital-acquired pathogens.
IS*Ecp1*	37 of 38 sites	S72c	Mobilization of extended-spectrum beta-lactamase (ESBL) genes, particularly *bla*CTX-M variants.
intI1_337	38 of 38 sites	S21c	Proxy for anthropogenic impact; facilitates resistance gene cassette assembly for multi-drug resistance.
IS*Aba3*	30 of 38 sites	S46c	Drives overexpression of OXA-type carbapenemase genes in *Acinetobacter baumannii*.
IS*6100*	38 of 38 sites	S21c	Highly active in forming composite transposons; frequently colocalizes with class 1 integrons.

4.3. Environmental drivers: diffuse system-wide failure, not discrete point sources

The strong negative correlation between DO and resistome abundance, and the equally strong positive correlation with NH_4-N, together imply that the main driver of the Akaki resistome is not attributable to any specific identifiable source. Widespread AMR in the watershed is not just due to hospitals, particular industrial outfalls, or single landfills, but is due to chronic, system-wide poor water quality due to inadequate waste management.

It is often tempting, and politically simpler, to frame the AMR problem in terms of controllable point sources: a hospital discharging inadequately treated effluent or a pharmaceutical manufacturer with non-compliant discharges. Such framings permit targeted enforcement actions and create clear accountability. However, data here show that such framing is insufficient for the Akaki catchment and, by extension, for comparable urban watersheds in the Global South with sanitation infrastructural problems. When a river system functions as an open sewer for millions of residents and industries, the entire river becomes the point source. Therefore, management response must be systemic and at the same spatial scale as the problem, i.e., system-wide improvements to waste containment and treatment, not arbitrary interventions at individual sites.

The enrichment of sulfonamide resistance downstream relative to Central sites adds a secondary dimension to this picture. Sulfonamide ARGs, particularly *sul1* and *sul2*, are strongly associated with both human wastewater and agricultural waste AMR selection (Karkman et al., 2019), and their downstream enrichment reflects the cumulative impact of agricultural runoff to already-impacted urban wastewater signals. This mixed downstream signature underscores that the watershed functions as a gradient integrator. ARG profiles at any given point reflect the cumulative contributions of all upstream sources, making the

downstream zone the legacy of all upstream pollution.

The utility of conventional water quality parameters as proxies for AMR risk is an important finding for practical surveillance design. DO and NH_4-N, often measured in water quality monitoring programs, emerge as strong predictors of resistome composition in places like Akaki watershed (Ott et al., 2021, Potgieter et al., 2020). This suggests that water quality data already being collected in many Global South cities might be leveraged immediately to identify AMR causal or risk hotspots without requiring other, more expensive analyses, enabling resource-limited agencies to prioritize where genetic surveillance and-or infrastructure investment might deliver the greatest risk reduction (Graham et al., 2018).

Furthermore, the prevalence of heavy metal resistance genes (e.g., *copA* and the *merA* spike at S40u) highlights metal-driven co-selection as a critical persistence mechanism (Berendonk et al., 2015). Persistent metals from mixed discharges exert continuous selective pressure, driving the co-retention of ARGs on shared MGEs even without direct antibiotic exposure. Mitigating AMR thus requires addressing both industrial and domestic effluents.

4.4. The Akaki as a model system for Global South urban watersheds

The infrastructural features that produce the resistome patterns documented here — inadequate WWTP coverage, effluents that increase rather than reduce pollutant loads relative to receiving waters, open defecation, and unmanaged livestock and industrial waste releases — are not unique to Addis Ababa. They characterize a broad and expanding class of rapidly urbanizing cities in sub-Saharan Africa, South Asia, and Latin America. Cities including Kampala, Uganda; Nairobi, Kenya; Dhaka, Bangladesh; Karachi, Pakistan; and Kathmandu, Nepal share WWTP coverage rates below 30-40% of urban households, similar land-use intensification trajectories, and comparable histories of water quality degradation driven by urbanization outpacing infrastructure investment (UNEP, 2023).

Where river AMR data exist from these comparable settings, the patterns are broadly consistent with what we report here. Studies of the Ganges and Yamuna rivers in India document ARG abundances of similar magnitude in urban reaches (Ahammad et al., 2014), driven by comparable combinations of domestic and industrial wastewater and agricultural runoff (Mittal et al., 2019, Yadav et al., 2021). Analysis of rivers in urban Kenya and South Africa has found comparable MGE diversity and ARG co-occurrence patterns linked to wastewater discharge (Abia et al., 2023). The globally synthesized river microbiome AMR assessment of Gao et al., (2024) found that river resistome magnitude was most strongly predicted by the scale of WWTP coverage and urban population density, exactly the factors that define the Akaki's problems. These convergent observations support the interpretation of Akaki as a model system rather than an outlier.

The implication is that the intervention logic derived from the Akaki data is similarly transferable. The primary target is not any specific resistance gene, pathogen, or point source; it is the underlying condition - the environmental “mixing bowl” of warm, hypoxic, nutrient-rich water carrying diverse microbial communities and their genetic cargo - that must be dismantled piece by piece. Expanding and upgrading wastewater management infrastructure is the highest-impact first intervention (Berendonk et al., 2015, UNEP, 2023), but it is not sufficient alone. Sewer containment, sewerage network improvement, reduced open defecation, better managed agricultural runoff, altered antibiotic use, and behaviour change around water contact are all needed components of a comprehensive response (UNEP, 2023). The spatial baseline provided by this study is a key foundation for designing, targeting, and evaluating those interventions.

4.5. Limitations and future directions

The single dry-season sampling campaign is an inherent constraint of this study, and it is important to be explicit about what this means for interpretation. The dry-season design was deliberate. Maximum pollutant concentrations occur during low-flow conditions and provide a picture of peak exposure conditions and clearer spatial signals for driver identification. Here these spatial patterns are supported by the statistical power of 38 concurrent sites spanning extreme pollutant gradients. However, wet-season dynamics will differ materially (Graham et al., 2011; Knapp et al., 2012): flash-flow dilution may temporarily reduce ARG concentrations at some sites, while stormwater-driven remobilisation of contaminated sediments may create transient hotspots elsewhere. Seasonal sampling campaigns are planned as the next phase of work, building directly on the spatial framework established here.

The reliance on HT-qPCR, while providing quantitative breadth across 309 ARGs and 57 MGEs simultaneously, does not provide the genomic context needed to confirm *in situ* gene transfer events, determine the genomic location of ARGs (chromosomal versus plasmid-borne), or trace precise host-gene associations. Long-read metagenomic sequencing of selected sites, particularly Central zone hotspots and along the hospital-proximate Kebena corridor, would substantially advance mechanistic understandings. Specific microbial source tracking (MST) markers were not employed in this campaign, but their inclusion in future work would enable more precise partitioning of human, animal, and agricultural AMR contributions to the overall resistome.

Despite these limitations, the dataset presented here provides among the most comprehensive quantitative characterizations of waterborne AMR in sub-Saharan Africa to date, and one of the most spatially detailed in the Global South. It establishes the baseline against which future interventions can be evaluated, identifies the environmental conditions and MGE families that most urgently require attention, and demonstrates the transferability of the analytical framework to comparable settings globally.

5. Conclusions

The Akaki River watershed represents an extreme hotspot for waterborne AMR, driven primarily by chronic, system-wide failures in waste containment that are common in dozens of rapidly urbanizing cities in the Global South. The spatial baseline established by this 38-site, 309-ARG, 57-MGE dataset reveals both the severity of that burden and, crucially, its mobilization potential. The pervasive co-occurrence of clinically important ARGs with broad-host-range conjugative plasmids (IncP-1α, IS*26*, IS*Ecp1*, *intI1*, IS*Aba3,* and IS*6100*) is broadly evident across waters routinely contacted by hundreds of thousands of residents. This suggests the Akaki is more than a passive conduit for AMR but an ecosystem where ARG transmission and evolution are almost certainly occurring at a catchment scale.

Dominant environmental AMR co-correlators, such as lower DO and elevated NH_4-N, are signatures of untreated waste loadings, which confirms that the Akaki problem is diffuse and systemic, not attributable to individual point sources. This argues for system-level interventions, such as expanding wastewater management coverage, improving sewer maintenance and containment, promoting alternatives to antibiotics, and reducing direct human and animal contact with impacted water. The correlation between conventional water quality parameters and resistome composition offers a practical and immediate option for AMR risk surveillance in resource-limited settings, enabling risk stratification without requiring detailed molecular biological analysis at every site.

The Akaki is not an outlier. Its conditions, including inadequate WWTP coverage, prevalent open defecation, a fragmented sewer infrastructure, and mixed domestic-agricultural-industrial pollution, are typical of urban watersheds across sub-Saharan Africa, South Asia, and Latin America. The resistome patterns documented here, and the environmental conditions that sustain them, will be recognizable to anyone working in comparable settings. The ultimate priority, both for Addis Ababa and across the Global South, is to progressively reduce physical and biological conditions that make their rivers mixing bowls for AMR

gene mobilization. The goal is to sever the pathways by which diverse microbial communities, ARGs, and MGEs co-mingle in water that humans and animals contact daily. Only with this scale of structural and other major changes will waterborne AMR and its risks in rapidly urbanizing regions be meaningfully reduced.

Declaration of Generative AI and AI-Assisted Technologies

During the preparation of this work the authors used Grok (xAI) to assist with generating and optimizing R code for figure preparation, and Gemini and Claude (Anthropic) to improve language, readability, and clarity of the manuscript. After using these tools/services, the authors carefully reviewed, edited, and verified all generated content and code, and take full responsibility for the accuracy, integrity, and originality of the published article.

CRediT authorship contribution statement

Yanyan Zhou: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Alemseged Tamiru Haile:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Mulugeta Kibret:** Writing – review & editing, Visualization, Formal analysis. **Hu Li:** Writing – review & editing, Visualization, Formal analysis. **Mihret Mersha Haileselassie:** Writing – review & editing, Investigation. **Tilaye Worku Bekele:** Writing – review & editing, Investigation. **Jemila Mohammed Kassa:** Writing – review & editing, Investigation. **Kirubel Demissie:** Writing – review & editing, Investigation. **Ethan R. Wyrsch:** Writing – review & editing, Investigation. **Steven P. Djordjevic:** Writing – review & editing, Investigation. **Kelly Jobling:** Writing – review & editing, Investigation. **Marcos Quintela-Baluja:** Writing – review & editing, Investigation. **Jian-Qiang Su:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Javier Mateo-Sagasta:** Writing – review & editing, Funding acquisition, Conceptualization. **David W Graham:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110554.

Data availability

Data will be made available on request.

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