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Detection of Antibiotic Resistance Genes in Urban Wastewater and Assessment of Ecological Risks

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الكشف عن جينات مقاومة المضادات الحيوية في مياه الصرف الصحي وتقييم المخاطر البيئية

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Abstract:
Urban wastewater carries microbes from human and animal sources, many of which harbor antibiotic resistance genes (ARGs). These genes can travel through sewer networks and treatment plants, reaching rivers, soil, and even returning to people. We review global approaches to detect ARGs in sewage and assess ecological risks from measured gene loads. We focus on three key methods: high-throughput qPCR panels, shotgun metagenomic sequencing, and culture with phenotypic screening. We compare their advantages, limits, and common practices. We compile evidence from large public datasets and case studies. Global sewage surveys (e.g. 79 cities, 60 countries) have revealed clear patterns of ARG abundance and diversity, linked to regional socioeconomic factors. We also examine how ARGs associate with mobile genetic elements (integrons, transposons, plasmids) within treatment plants and the wider environment. We then link measured ARG loads to risk thresholds. We discuss published Predicted No Effect Concentrations (PNECs) for antibiotic selection (e.g. 8 ng/L–64 µg/L) and a 0.05 µg/L default PNEC for screening. We outline major exposure routes (effluent, biosolids, overflows, aerosols) and propose a tiered monitoring strategy. This strategy blends rapid qPCR panels with targeted metagenomics and risk screening against PNECs. We include figures (maps, networks, heatmaps) and tables summarizing methods and thresholds. Key gaps remain, such as better tracking of mobile elements and fate modeling. Our review supports wastewater-based AMR surveillance as a practical tool and provides guidance for risk assessment and management.

Keywords: antibiotic resistance genes, wastewater, metagenomics, qPCR, ecological risk, surveillance.

1. Introduction

Antimicrobial resistance is a growing global health threat. Tracking ARGs in human populations is challenging because clinical sampling is costly and incomplete. Urban wastewater acts as a pooled sample reflecting large populations, capturing human gut bacteria and other microbes without individual consent issues. Sewage-based monitoring has shown promise as an efficient and ethical surveillance method. For example, Hendriksen *et al.* (2019) analyzed untreated sewage from 79 sites in 60 countries to characterize ARGs using metagenomics. They found strong regional differences: Europe, North America and Oceania had lower ARG loads and different gene profiles than Africa, Asia, and South America. ARG abundance also correlated with socioeconomic and sanitation factors. Other studies expanded these surveys: Munk *et al.* (2022) sequenced sewage from 243 cities in 101 countries and similarly saw clear regional resistome patterns driven in part by bacterial community differences. Moreover, sewage resistome data can partly predict clinical resistance trends when combined with local context. Karkman *et al.* (2020) showed that the sewage ARG signal correlated with *E. coli* clinical

resistance rates, especially when adding socioeconomic data. These findings suggest that wastewater profiling can complement traditional surveillance, especially where clinical data are scarce.

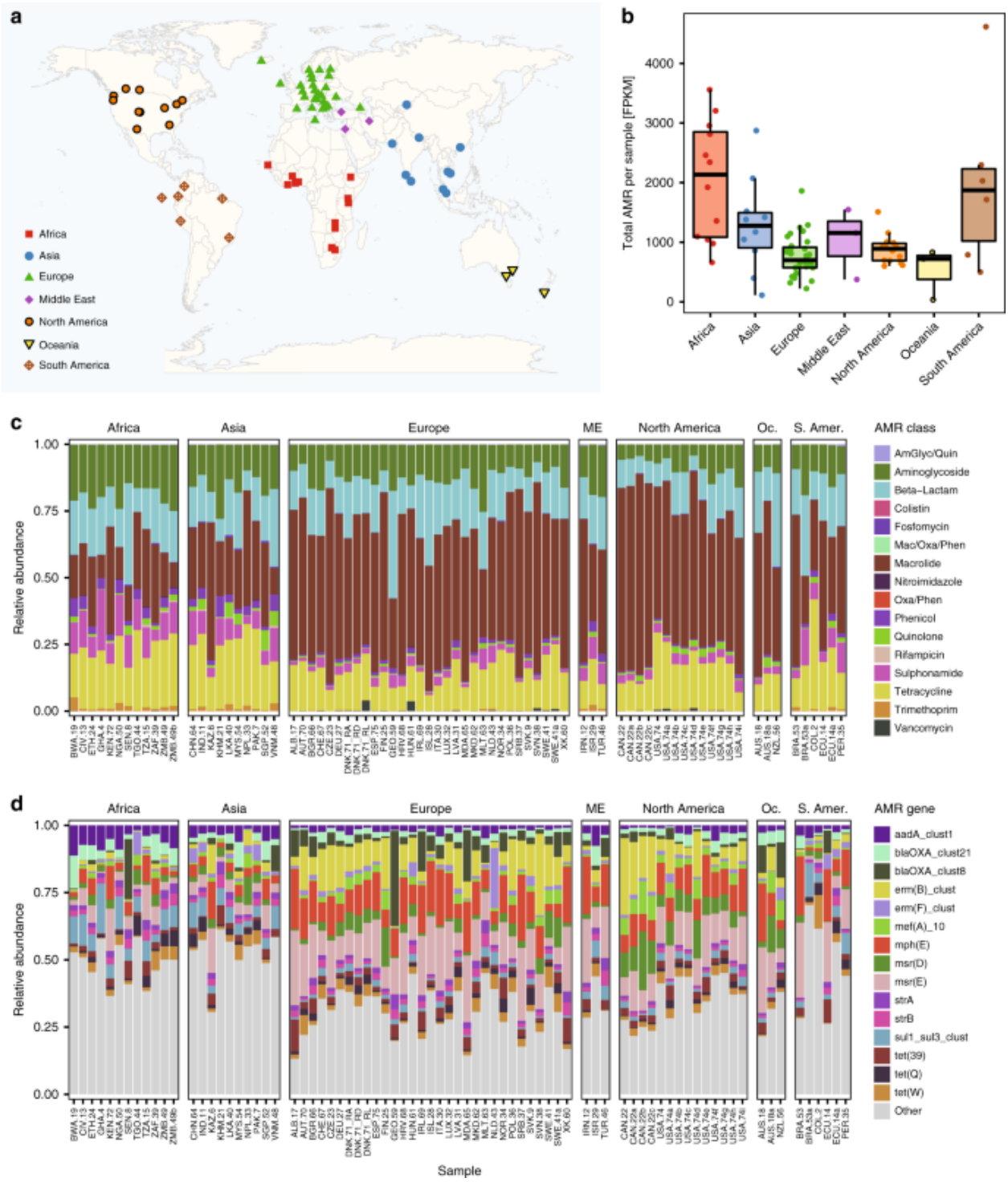

Figure 1 Global sewage sampling sites and AMR overview (map of sites and resistome composition by class). Source: Hendriksen et al. (2019).

Wastewater treatment plants reduce the number of viable bacteria, but they cannot entirely eliminate ARGs or their carriers. ARGs often reside on mobile genetic elements (MGEs) like plasmids and integrons that survive treatment. These MGEs can facilitate ARG spread in downstream rivers, soils, or even back to humans. Many ARGs persist in treated effluent and sludge, posing risks for environmental selection and transmission. In this context, assessing the ecological risk of ARG release requires linking measured gene loads to selection pressure from antibiotic residues, as well as understanding exposure

pathways. This review compiles methods, data, and risk frameworks for ARG monitoring in urban wastewater and evaluates potential ecological risks. We aim to provide practical guidance for surveillance, risk assessment, and mitigation.

2. Methods to Detect ARGs in Urban Wastewater

2.1 High-Throughput qPCR Panels

High-throughput quantitative PCR (qPCR) can target hundreds of known ARGs and integron genes simultaneously. Custom primer panels (often 300–400 assays) allow parallel screening of many targets in each sample. The assays yield absolute or relative abundances of specific genes with good sensitivity (detection down to ~10–100 copies per reaction). Results are usually normalized to a reference gene (e.g. 16S rRNA or *crAssphage*) to account for bacterial load or sample volume. Care is taken to check for PCR inhibitors in wastewater. The main strengths are speed, cost-effectiveness, and comparability across studies. qPCR panels are well suited for routine monitoring and identifying high-risk sites. Their limitations are that they can only detect the chosen set of known genes. They cannot capture novel or unexpected ARGs. Thus, qPCR provides a focused snapshot of known resistance markers, useful for trends and hotspot screening.

Table 1 Detection methods used in public datasets.

Method	Typical targets	Detection limit	Strengths	Limits	Representative study
High-throughput qPCR	~300–400 ARGs + intI1	~10–100 copies/reaction	Rapid, quantitative, comparable	Targets are pre-defined; no novel ARGs	You et al. (2019)
Shotgun metagenomics	All DNA reads (all ARGs)	Depth-dependent	Broad, retrospective analysis	Expensive; short reads limit context	Hendriksen et al. (2019); Munk et al. (2022)
Culture + AST + WGS	Viable pathogens	No gene limit (culture-based)	Links genotype and phenotype	Only cultivable organisms; labor-intensive	Flach et al. (2021)

2.2 Shotgun Metagenomics

Shotgun metagenomic sequencing analyzes all DNA in a wastewater sample. It profiles the complete resistome, the taxonomic community, and mobile elements in one run. Sequencing reads are mapped to reference databases of ARGs and MGEs. This approach can also discover new or variant genes by assembly and homology. Metagenomics is powerful for large surveys and global comparisons because raw sequence data can be re-analyzed as databases improve. The drawbacks are higher cost and required depth of sequencing. Wastewater is highly diverse, so deep sequencing is needed to detect low-abundance genes. Short-read data often cannot link ARGs to their host organisms or mobile elements with certainty. Emerging methods like hybrid assembly or chromosome conformation capture (Hi-C) can help connect genes to hosts, but they are not routine yet. Overall, metagenomics gives a broad, unbiased view but may miss very rare genes and struggles with gene context.

2.3 Culture and Phenotypic Screens

Culturing approaches enrich viable antibiotic-resistant bacteria (ARB) from wastewater. Samples are plated on selective media containing antibiotics, and resistant colonies are counted or tested. Isolates can be subjected to antimicrobial susceptibility testing (AST) and whole-genome sequencing to link phenotypes with genotypes. This provides clinically relevant information and identifies pathogens. However, most environmental microbes cannot be easily cultured. Culture methods also sample far fewer taxa than metagenomics. They may miss non-culturable ARG carriers. For example, Flach *et al.* (2021) collected hospital sewage and grew bacteria on carbapenem-selective agar to quantify carbapenem-resistant Enterobacterales. They combined this with qPCR detection of carbapenemase genes and genomic typing of isolates. Such studies show the value of culture in confirming specific threats, but they are labor-intensive and not comprehensive. In practice, culture-based surveillance is best used alongside genetic screening methods.

3. Publicly Available Experimental Datasets

3.1 Global Sewage Metagenomes

Two landmark studies provide open datasets of sewage resistomes. Hendriksen *et al.* (2019) collected untreated sewage from 79 sites in 60 countries and applied standardized shotgun metagenomic analysis. They reported regional differences and linked ARG levels to socioeconomic indicators. Their data include relative abundances of ARG classes for each site. A follow-up by Munk *et al.* (2022) expanded the sampling to 243 cities in 101 countries. This “Big Data” resistome effort also used metagenomics and revealed clear continental patterns. Munk *et al.* found that ARGs cluster by region and by antibiotic class, partly reflecting local bacterial taxa. They also studied the genetic contexts of common ARGs and noted certain regions might be hotspots for gene transmission. These global sewage surveys are publicly available and have become references for comparing local data.

3.2 Mobile Resistome within Treatment Plants

Several studies have examined how MGEs move ARGs across environments and wastewater plant compartments. In one notable study, You *et al.* (2019) used long-read Nanopore sequencing alongside Illumina to analyze influent, activated sludge, and effluent in multiple WWTPs. They found most detectable ARGs were located on plasmids. Class 1 integrons and other transposons were also common on plasmids, highlighting their role in gene spread. Integrative and conjugative elements carrying multiple ARG types were found across plants. The same study tracked the hosts of ARGs and noted a diversity of resistant bacteria in the final effluent, implying a high potential for environmental dissemination.

3.3 Sewage and Clinical Resistance Links

Connections between sewage resistomes and clinical antibiotic resistance have been explored. Karkman *et al.* (2020) compared sewage ARG data to clinical surveillance of invasive *E. coli* across multiple countries. They found that overall sewage ARG abundance correlated with national *E. coli* resistance prevalence. However, no single metric perfectly predicted resistance for all antibiotic classes. The predictive power increased when socioeconomic factors (e.g. sanitation, income) were added to the model. This indicates that sewage metagenomics provides useful signals but is affected by local context. Overall, studies suggest sewage can track broad resistance trends, especially when combined with public health data, but it cannot yet replace detailed clinical surveillance on its own.

3.4 Hospital Sewage as Early Warning

In settings with low clinical prevalence of certain resistances, hospital sewage can act as an early-warning system. Flach *et al.* (2021) monitored sewage from a tertiary hospital in Sweden for carbapenemase-producing *Enterobacterales* (CPE) over two years. They used qPCR for key carbapenemase genes and cultured resistant strains. For three genes (bla_NDM, bla_OXA-48-like, bla_KPC), sewage levels corresponded with patient cases. Notably, a rise in OXA-48-like gene counts in sewage preceded the detection of cases in patients by a few months. They also recovered matching strains from sewage and patient samples for NDM producers. Their conclusion was that sewage surveillance shows “both promise and limitations” as a complement to clinical monitoring. Such studies indicate that focused sewage monitoring (especially from hospitals) can flag emerging threats before they appear widely in clinics.

4. Results from the Literature Synthesis

4.1 Global Abundance and Diversity Patterns

Resistome surveys reveal that ARG levels vary greatly by region. Global data show the highest ARG abundances in parts of Africa, Asia, and South America, and much lower levels in Europe, North America, and Oceania.

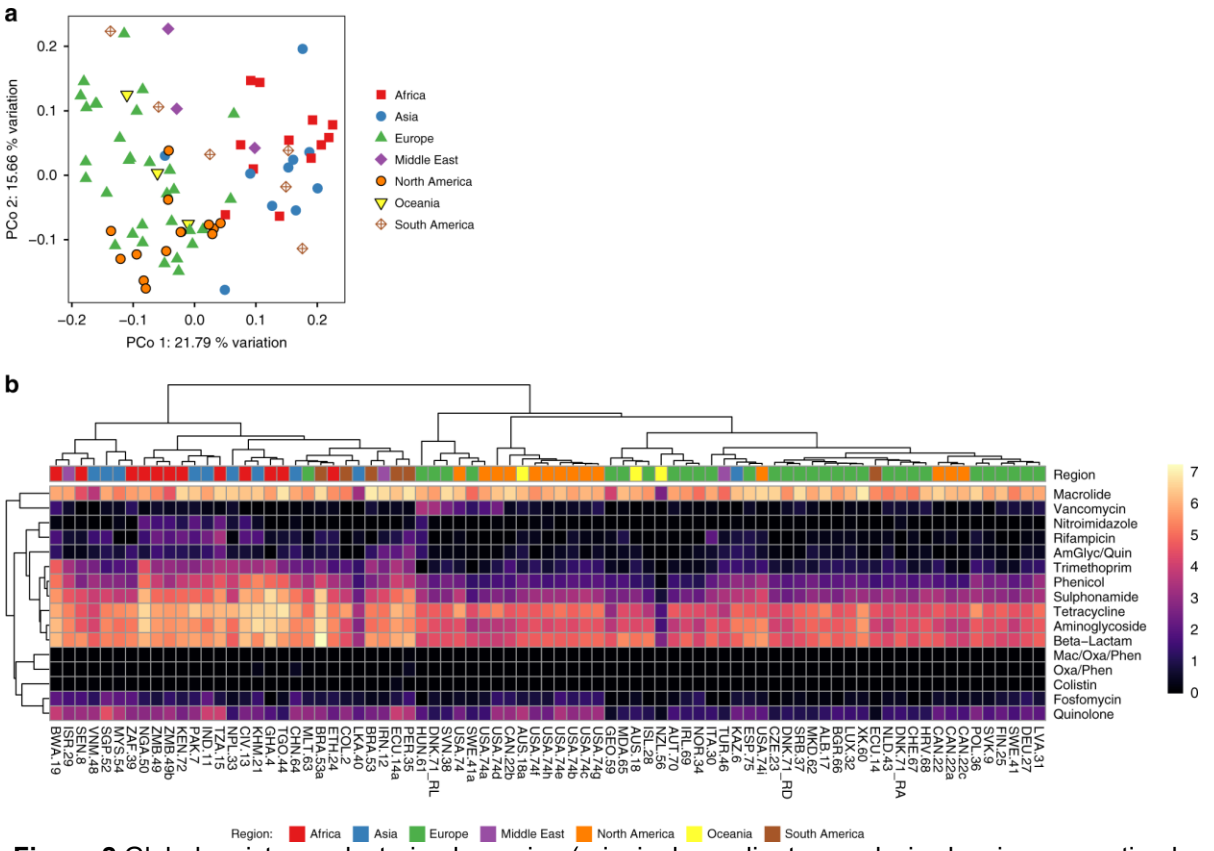

Figure 2 Global resistome clustering by region (principal coordinates analysis showing separation by continent). Source: Hendriksen et al. (2019).

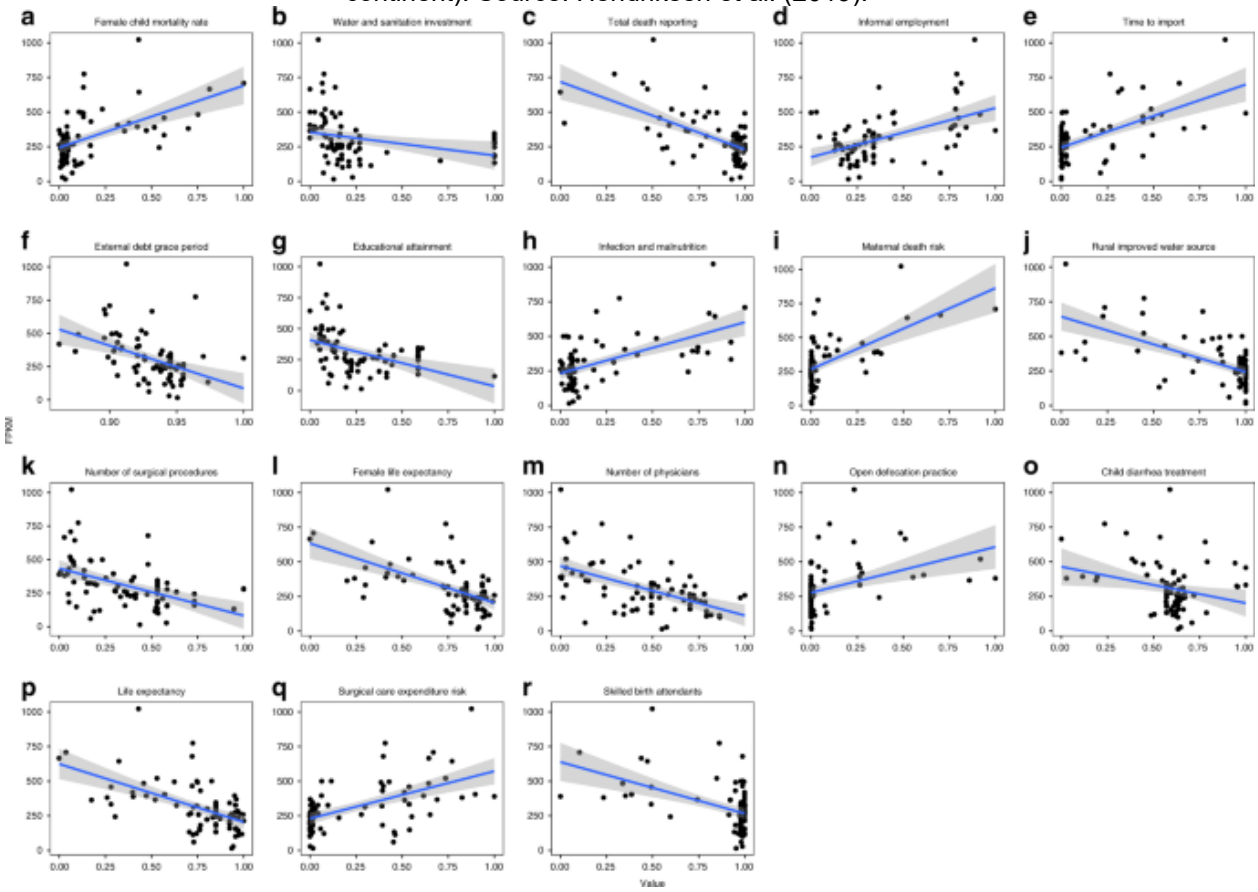

Figure 3 Global resistome heat map and diversity (heatmap of ARG class abundance across countries). Source: Hendriksen et al. (2019).

For example, Hendriksen *et al.* found African sites had the greatest ARG fragments per kilobase per million (FPKM). They also reported compositional differences: macrolide resistance genes (e.g. *mefA*) dominate in many high-income areas, while sulfonamide or phenicol resistance genes are more abundant in lower-income regions. Socioeconomic, health, and sanitation factors explained a large fraction of this variation.

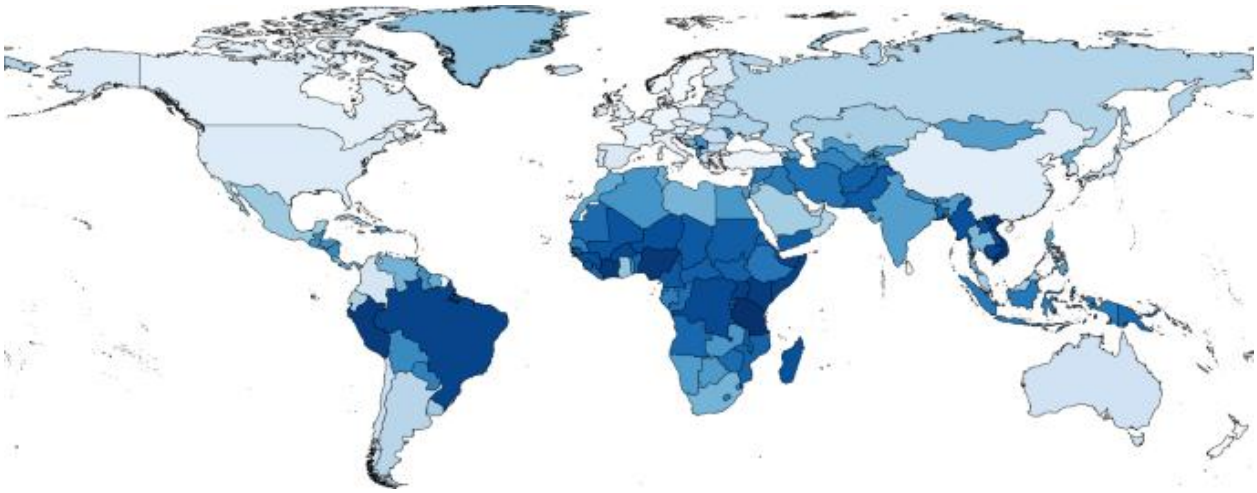

Figure 4 Top ARG contributors across regions (bar chart of most abundant ARGs in sampled cities). Source: Hendriksen et al. (2019).

Munk *et al.* similarly found distinct regional clusters in resistomes across their 101-country dataset.

4.2 Mobile Elements Shape the Wastewater Resistome

Mobile genetic elements (MGEs) strongly influence the wastewater resistome. Class 1 integrons, transposons, and plasmids carry many of the ARGs found in sewage and allow genes to jump between species. For example, You *et al.* demonstrated that most ARGs in WWTP compartments were plasmid-borne, and integrons and transposons had higher prevalence on plasmids than chromosomes. Network analyses from this and related studies show broad sharing of ARGs across diverse bacteria in sewer and sludge communities. This widespread mobility raises concern because it means even if treatment kills many bacteria, MGEs can persist and transfer ARGs to new hosts downstream. In effect, the wastewater environment acts as a mixing ground, accelerating horizontal transfer of resistance genes.

4.3 Treatment Performance and Residual Risk

Conventional activated sludge treatment removes a large portion of live bacteria, but it is less effective at removing ARGs from the system. Even after disinfection, treated effluent may still contain extracellular DNA and dead-cell debris carrying ARGs. You *et al.* noted that a wide variety of antibiotic-resistant bacteria survived into the final effluent of treatment plants. While viability is reduced, residual ARGs (e.g. *intI1* and *sul1*) often persist at measurable levels. These residual genes in effluent (and in biosolids) can pose a risk if conditions downstream allow their uptake and propagation. For example, if antibiotic residues or co-selective metals are present, they could select for bacteria that acquire the released ARGs. Thus, treatment lowers but does not eliminate AMR risk. Disinfection step design and sludge management are important risk control points. Monitoring studies consistently find that treated wastewater still carries enough ARGs to warrant concern about environmental dissemination.

4.4 Links to Clinical Resistance

Evidence supports a link between sewage resistomes and broader clinical resistance patterns. In general, countries with higher sewage ARG loads tend to report higher antibiotic resistance rates in human infections. Karkman *et al.* found that sewage ARG counts (particularly *intI1* integron gene) were significantly associated with clinical *E. coli* resistance levels. Importantly, combining sewage data with socioeconomic indicators improved predictions of clinical resistance. This means sewage-based measures can reflect the general resistance burden in a population, though they lack resolution to predict specific antibiotic-by-antibiotic prevalence. Therefore, sewage profiling can serve as a useful adjunct to clinical surveillance, especially for broad trends and emerging threats (e.g. a rise in a critical ARG in sewage might prompt targeted clinical testing). The current limit is that sewage data alone cannot yet replace patient isolate data, but it adds valuable context and early warnings.

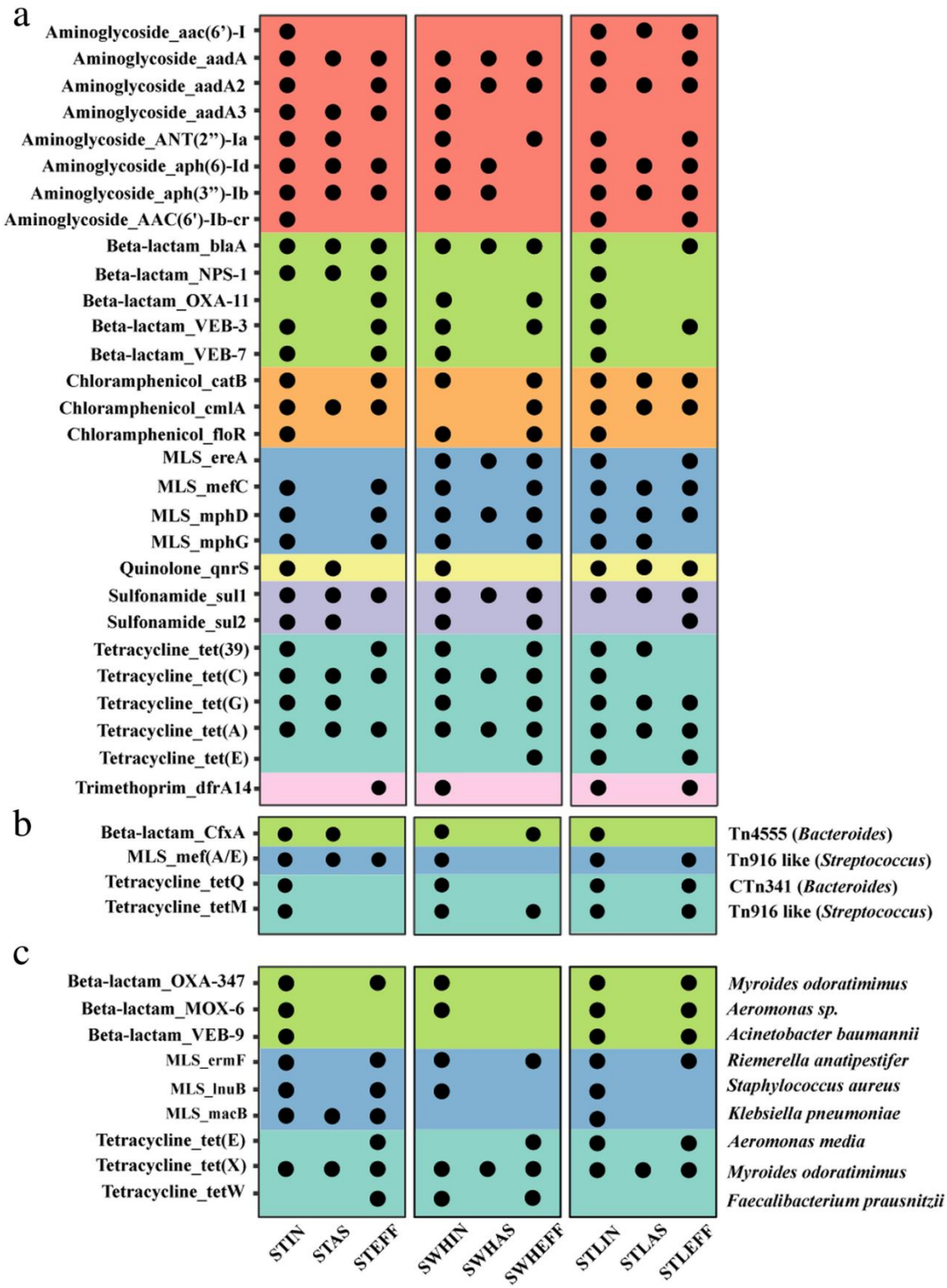

Figure 5 Mobile resistome networks in wastewater (network diagram of ARGs shared across taxa and sites). Source: You et al. (2019).

5. Risk Assessment Framework

5.1 Selection-Based Thresholds

Antibiotic residues in wastewater can select for resistance even at low concentrations. To guide risk assessment, researchers have proposed ecological thresholds known as PNECs (Predicted No Effect Concentrations) for resistance selection. Bengtsson-Palme & Larsson (2016) used MIC data to estimate PNECs below which antibiotics should not enrich resistance. Their computed PNECs ranged from about 8 ng/L to 64 µg/L across 111 common antibiotics. These values often lie below known ecotoxicity

thresholds, reflecting the sensitivity of microbial selection. For antibiotics lacking data, Vestel *et al.* (2022) recommend a default PNEC of 0.05 µg/L.

Table 2 Screening thresholds for ecological risk of resistance selection.

Antibiotic assessment approach	Threshold guidance	Notes	Reference
Drug-specific PNEC for AMR selection	Use published PNEC per antibiotic	Derived from lowest MIC and selection data	Bengtsson-Palme & Larsson (2016)
Default PNEC when data lacking	0.05 µg/L	Conservative screening value	Vestel et al. (2022)

This conservative screening value can be used for initial risk flags. Applying these PNECs means measuring antibiotic concentrations in effluents and comparing them to thresholds. If levels exceed the PNEC, there is concern for resistance selection. When below PNECs, the risk of promoting AMR is judged low. This selection-based risk framework provides a quantitative way to link chemical measurements to AMR outcomes.

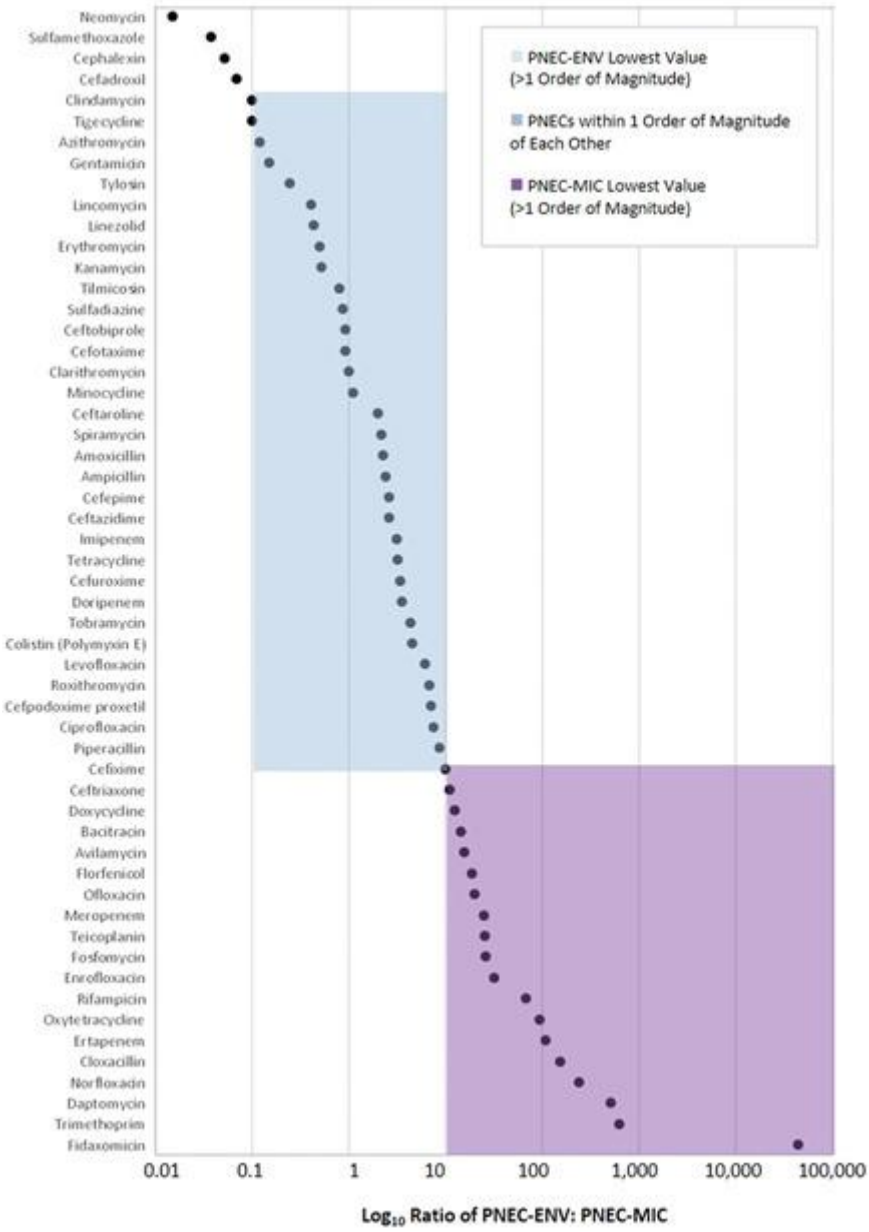

Figure 6 Selection-protective thresholds comparison (log-ratio of environmental PNECs vs. MIC-based PNECs across antibiotics). Source: Vestel et al. (2022).

5.2 Exposure Pathways

Multiple environmental routes can expose humans and ecosystems to wastewater ARGs and residues. Effluent discharges to rivers and lakes spread ARGs and any residual antibiotics into aquatic ecosystems. Biosolids applied to land as fertilizer can introduce ARGs and antimicrobials to soils and crops. Combined sewer overflows (CSOs) during storms release untreated sewage directly. Aerosols generated at treatment plants may carry ARGs into air, potentially affecting plant workers or nearby residents. Each pathway has distinct controls: improving effluent treatment and limiting antibiotic release addresses effluent risk; regulated land application and sludge treatment mitigate soil exposure; CSO control requires infrastructure upgrades; aerosol exposure can be reduced by filters or moving units indoors. Recognizing these routes is essential for comprehensive risk management of wastewater AMR.

5.3 A Tiered Risk Screen

A practical monitoring plan may use a tiered approach. Tier 1: Screen key genetic markers by qPCR in influent and effluent. For example, measure total ARG load (16S-normalized), the class 1 integron gene *intI1*, and a selection of clinically relevant ARGs (e.g. *blaCTX-M*, *blaNDM*, *mcr*). Include an indicator of human fecal pollution (e.g. *crAssphage*) to contextualize human contribution. Tier 2: Analyze effluent chemistry. Measure concentrations of relevant antibiotics and compare to PNECs. Flag any compounds above thresholds. Tier 3: In sites or times of concern, perform deeper analysis. This could be shotgun metagenomics or targeted sequencing for detailed resistome profiling, including MGEs. Also monitor for specific pathogen markers if justified. Tier 4: Combine these findings with exposure assessment (e.g. hydrology, land use) and clinical data. Decide on risk management actions (e.g. treatment upgrades, hospital surveillance) accordingly. This tiered strategy aligns scientific monitoring with practical action, using simple qPCR screens for routine surveillance and reserving resource-intensive methods for critical situations.

6. Recommended Monitoring Panel

Based on literature and practice, we suggest a core panel of targets for routine wastewater AMR monitoring:

- **16S rRNA gene:** for total bacterial load and data normalization.
- **crAssphage gene:** a marker of human fecal pollution, helping distinguish human sources.
- **intI1:** class 1 integron integrase gene, a proxy for anthropogenic ARG load and horizontal gene transfer potential.
- **sul1:** sulfonamide resistance gene, often on class 1 integrons (ubiquitous and indicative of treated wastewater).
- **bla_{CTX-M} (e.g. CTX-M-15 variant):** common extended-spectrum β-lactamase in Enterobacterales, high clinical relevance.
- **bla_{NDM}:** carbapenemase gene of critical concern in carbapenem-resistant pathogens.
- **mefA:** macrolide resistance gene, chosen for Europe/N. America where macrolide use is high.
- **tetM:** tetracycline resistance gene, widespread and often mobile.
- **mcr-1:** plasmid-mediated colistin resistance gene.

Including these targets covers major antibiotic classes and mobility markers. Controls (e.g. artificial spikes) and inhibition tests should accompany qPCR. This set aligns with markers used by Hendriksen (2019), You (2019), and others. It can be adjusted based on local antibiotic usage patterns or emerging threats.

9. Discussion

Wastewater surveillance is a powerful approach for AMR tracking. It samples entire communities without the biases of clinical sampling. Our synthesis shows that global sewage data reveal consistent geographic patterns of ARG pollution aligned with development and sanitation metrics. Sewage resistomes also capture signals of clinical resistance trends, though they require context data for accurate interpretation. For ecological risk assessment, we stress combining gene and chemical data. ARG detection alone does not prove selection is occurring. It must be paired with antibiotic concentration measurements and PNEC thresholds. Published antibiotic-specific PNECs (from MIC

data) can flag risky levels. In the absence of compound-specific data, the default PNEC of 0.05 µg/L provides a useful guardrail.

Control efforts should target both genes and antibiotics. Improving wastewater treatment (especially tertiary processes) can reduce ARB and ARG release. Limiting combined sewer overflows and other bypasses will cut raw sewage inputs. Disinfection (chlorine, UV, etc.) can lower viable cells, but efforts are needed to degrade extracellular DNA and antibiotics as well. At the source, judicious antibiotic use in healthcare and agriculture will reduce selection pressures entering wastewater. In particular, attention to mobile elements is warranted: reducing release of bacteria carrying class 1 integrons or multidrug plasmids can blunt the environmental spread of resistance.

Public health actions can benefit from wastewater AMR data. In resource-limited settings without routine clinical labs, sewage surveillance can offer early warnings of rising resistance. For example, detecting a surge of a critical ARG (e.g. *blaNDM* or *mcr*) in city sewage could prompt targeted clinical screening or stewardship efforts. Even in well-monitored regions, sewage data provide an independent check on official reports and can identify disparities between hospital and community resistance. As shown by Karkman *et al.*, combining sewage data with socioeconomic indicators yields robust resistance prevalence models, suggesting that one day large-scale sewage monitoring might forecast regional resistance trends on its own.

10. Limitations and Future Work

Several challenges remain. Short-read metagenomics often fails to link ARGs to specific hosts or plasmids, which hampers risk interpretation. Emerging long-read and single-cell techniques may help resolve this. Current qPCR panels miss novel or unexpected genes, so they need periodic updating as new ARGs emerge. Analytics for antibiotics and other co-selective agents in wastewater lag behind gene analysis; more chemical monitoring is needed globally. Harmonized reporting of ARG data (e.g. gene copies per liter, per biomass) would improve cross-study comparisons. Finally, fate and transport models for ARGs (including MGEs) are underdeveloped. Such models would connect measured outputs (effluent concentrations) to exposure risks (e.g. potential human uptake). Research in these areas mobile element tracking, integron dynamics, modeling of resistance spread is crucial to advance environmental AMR risk assessment (see Larsson & Flach 2022 for a broad review of these gaps).

Conclusions

Urban wastewater provides a powerful lens into community-level antibiotic resistance. High-throughput methods like metagenomics and multiplex qPCR can rapidly profile the collective resistome. Global data show clear geographic patterns and broad links to clinical resistance. Effective risk assessment must go beyond gene counts; it should integrate antibiotic concentration thresholds (PNECs) to predict where selection may occur. We outline a tiered monitoring strategy that combines ARG screening, chemical analysis, and exposure assessment. This approach can guide wastewater management (treatment upgrades, monitoring) and public health actions. By targeting both the spread (genes) and drivers (antibiotics), we can better manage environmental selection for resistance. Ongoing research on mobile genes, long-read sequencing, and AMR fate models will further refine our ability to use sewage surveillance to protect both human and ecosystem health.

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