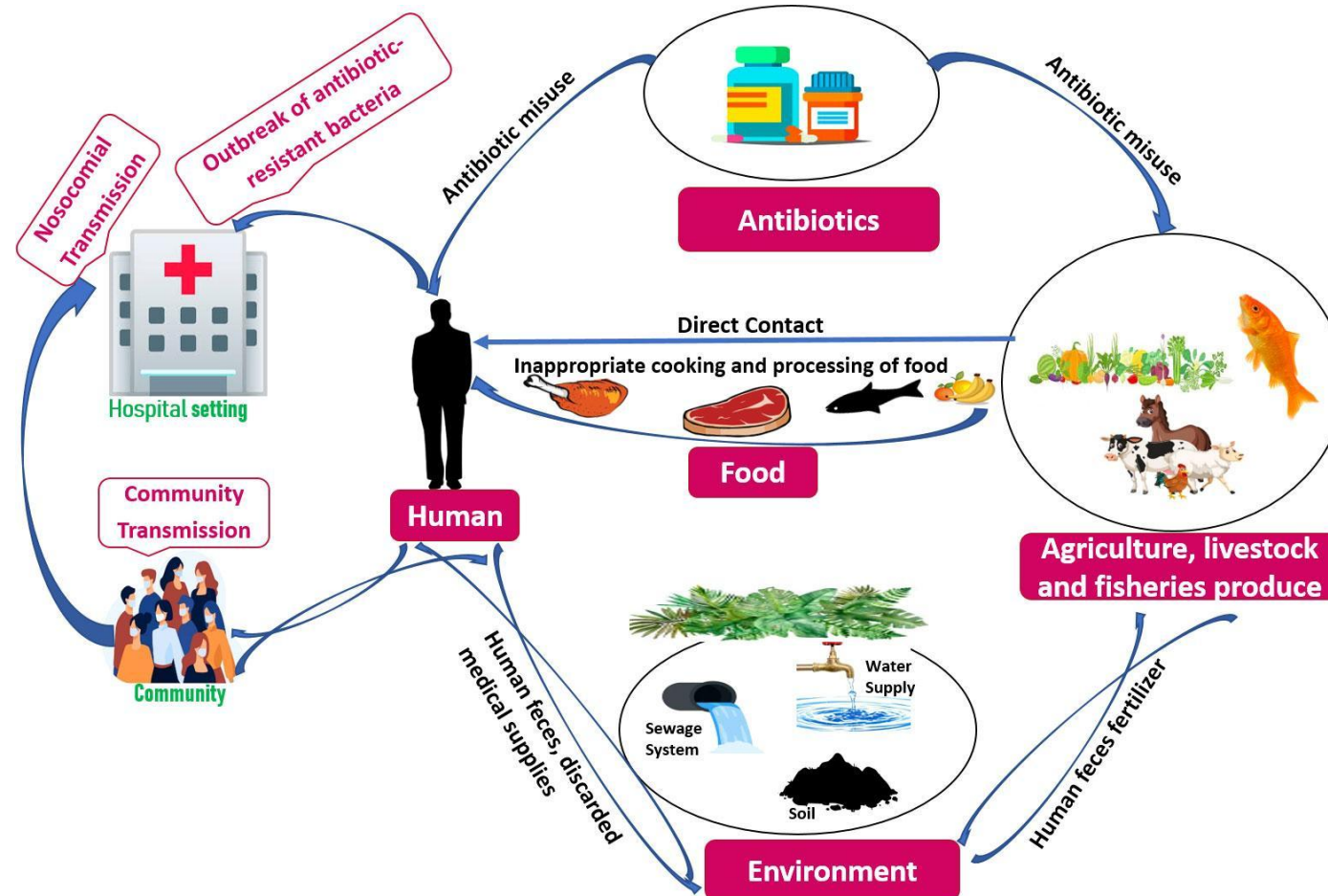

WASTEWATER SURVEILLANCE FOR AMR CONTROL: BUILDING A ONE HEALTH EARLY WARNING SYSTEM

DR AFREENISH HASSAN
PAKISTAN

AMR: A ONE HEALTH CHALLENGE



CURRENT AMR SURVEILLANCE: GAP

Primarily clinical/healthcare-based

Dependent on patients accessing healthcare

Diagnostic and geographic gaps

Limited community/environmental visibility

Question:

Can we detect AMR at the population and environmental level?

WHY WASTEWATER?

Wastewater = population-level biological sample

It can provide

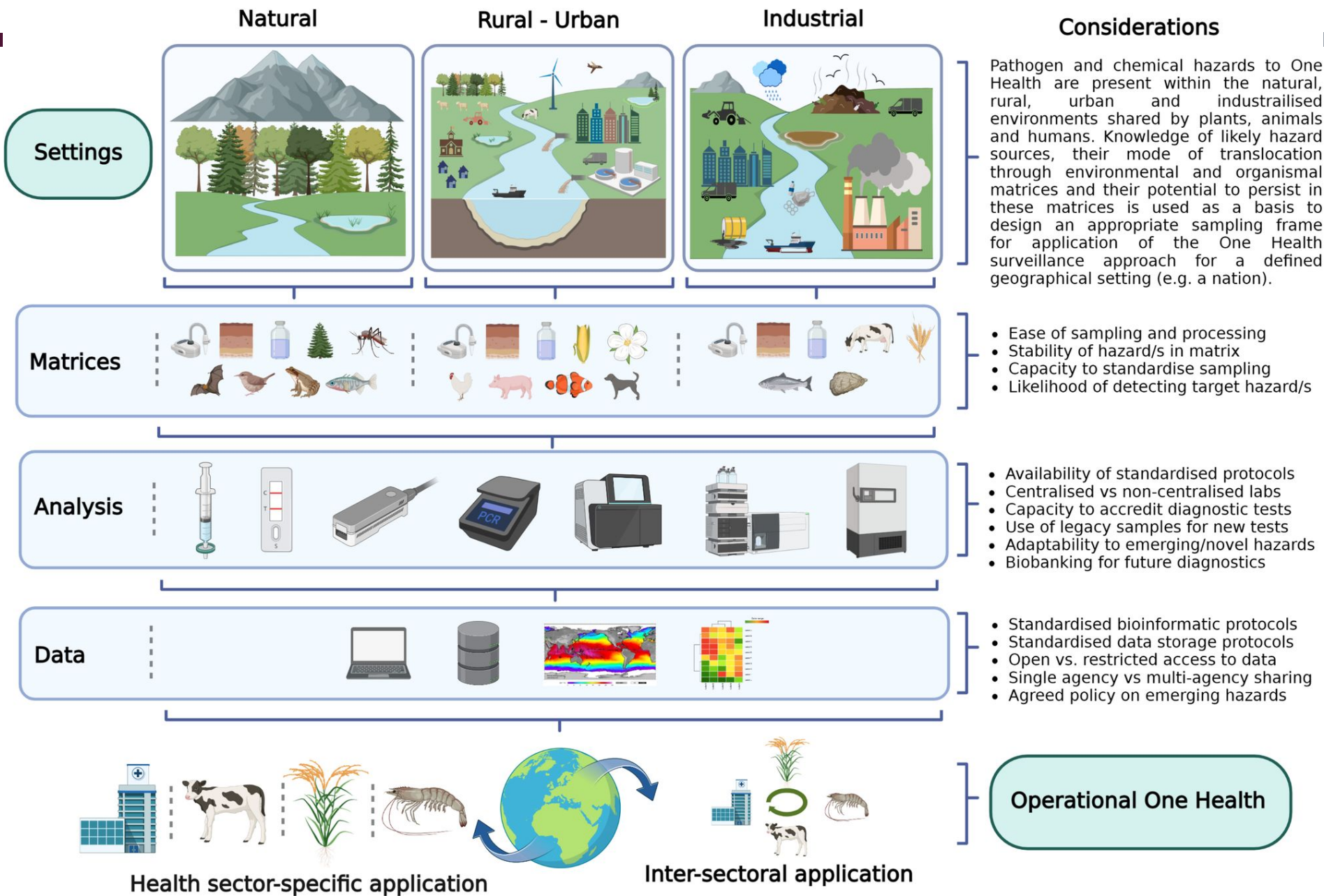
- Population-integrated information
 - Spatial and temporal trends
 - Non-invasive surveillance
- Continuous/repeated monitoring
- Signals from community and healthcare settings

Wastewater surveillance has the potential to provide an additional population-level layer of AMR intelligence and early warning, but interpretation requires integration with clinical, epidemiological and genomic surveillance



WHAT CAN WASTE WATER SURVEILLANCE DETECT RELATED TO AMR?

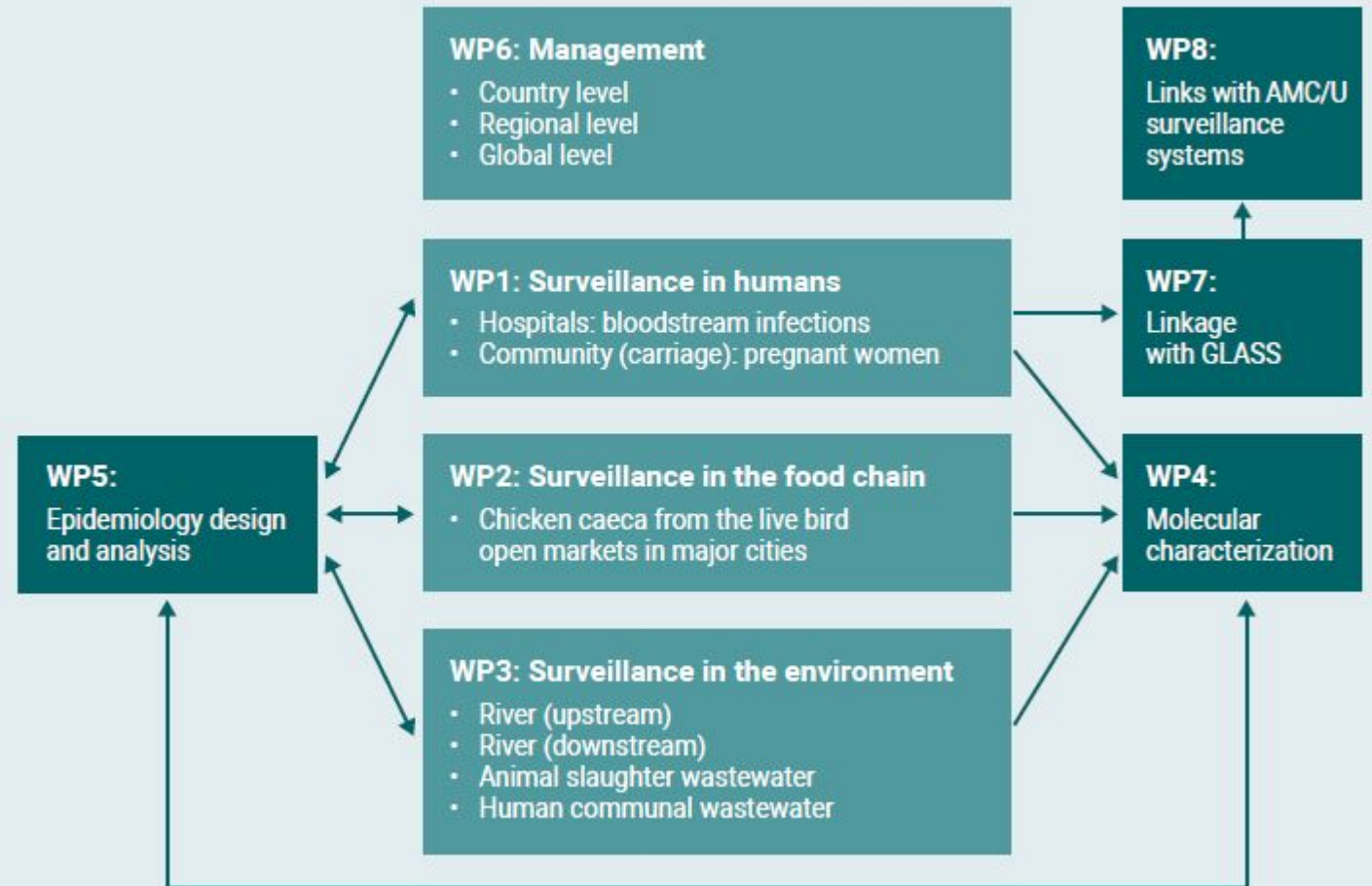
AMR Target	What it tells us
Resistant organisms	Circulation/prevalence
Antibiotic Resistant Genes	Resistance determinants
Specific clones	Emerging threats/transmission
Genomes	Diversity and evolution
Trends	Increasing/decreasing signals



WHO integrated global surveillance on ESBL-producing *E. coli* using a “One Health” approach (2021)

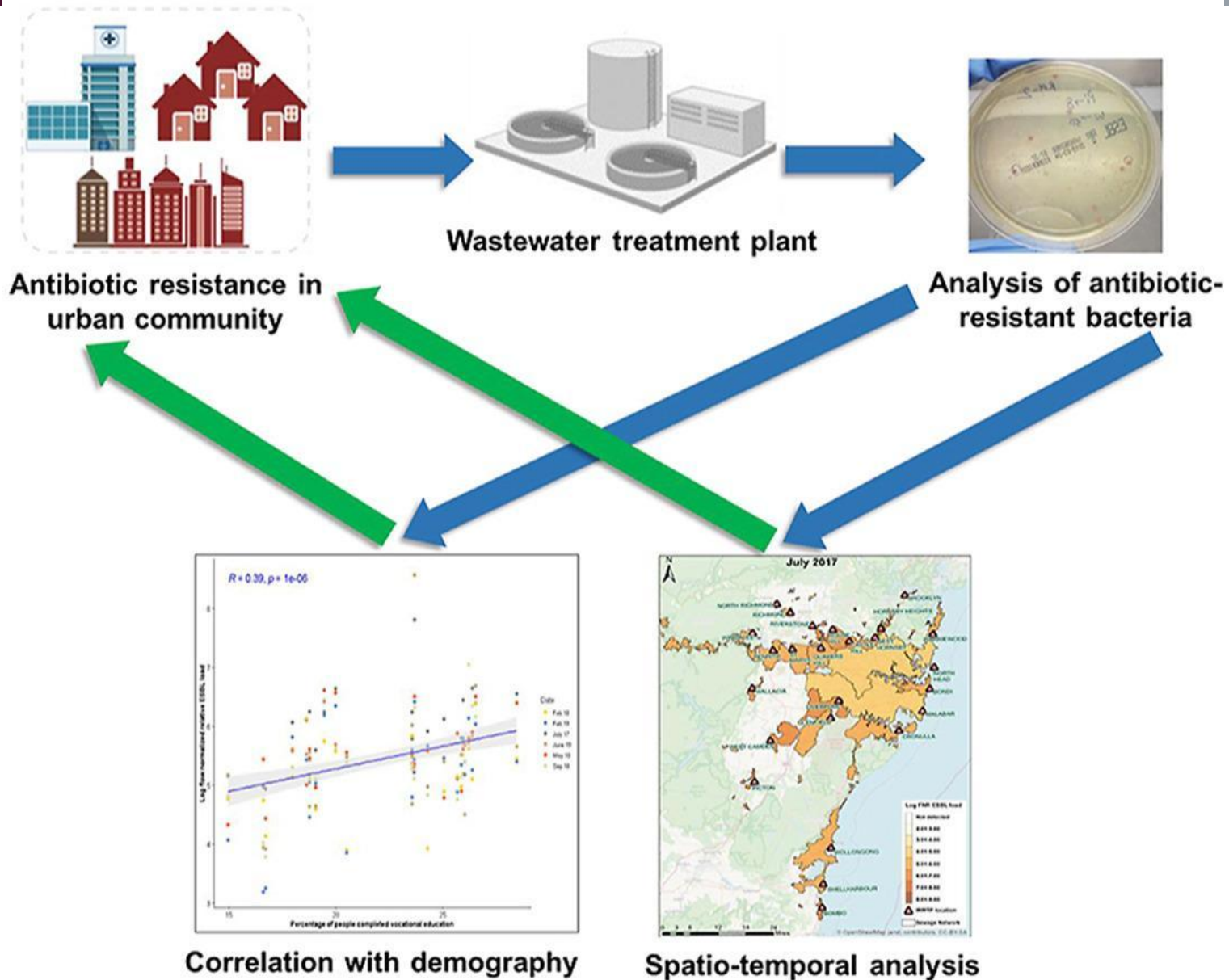
The World Health Organization (WHO) Tricycle protocol proposes integrated surveillance protocols for a single indicator, extended spectrum β -lactamase (ESBL) producing *Escherichia coli*, i.e. in wastewater and surface water

Figure 1.1 ESBL-Ec Tricycle structure



COMMUNITY WASTEWATER SURVEILLANCE FOR AMR: EVIDENCE FROM GREATER SYDNEY

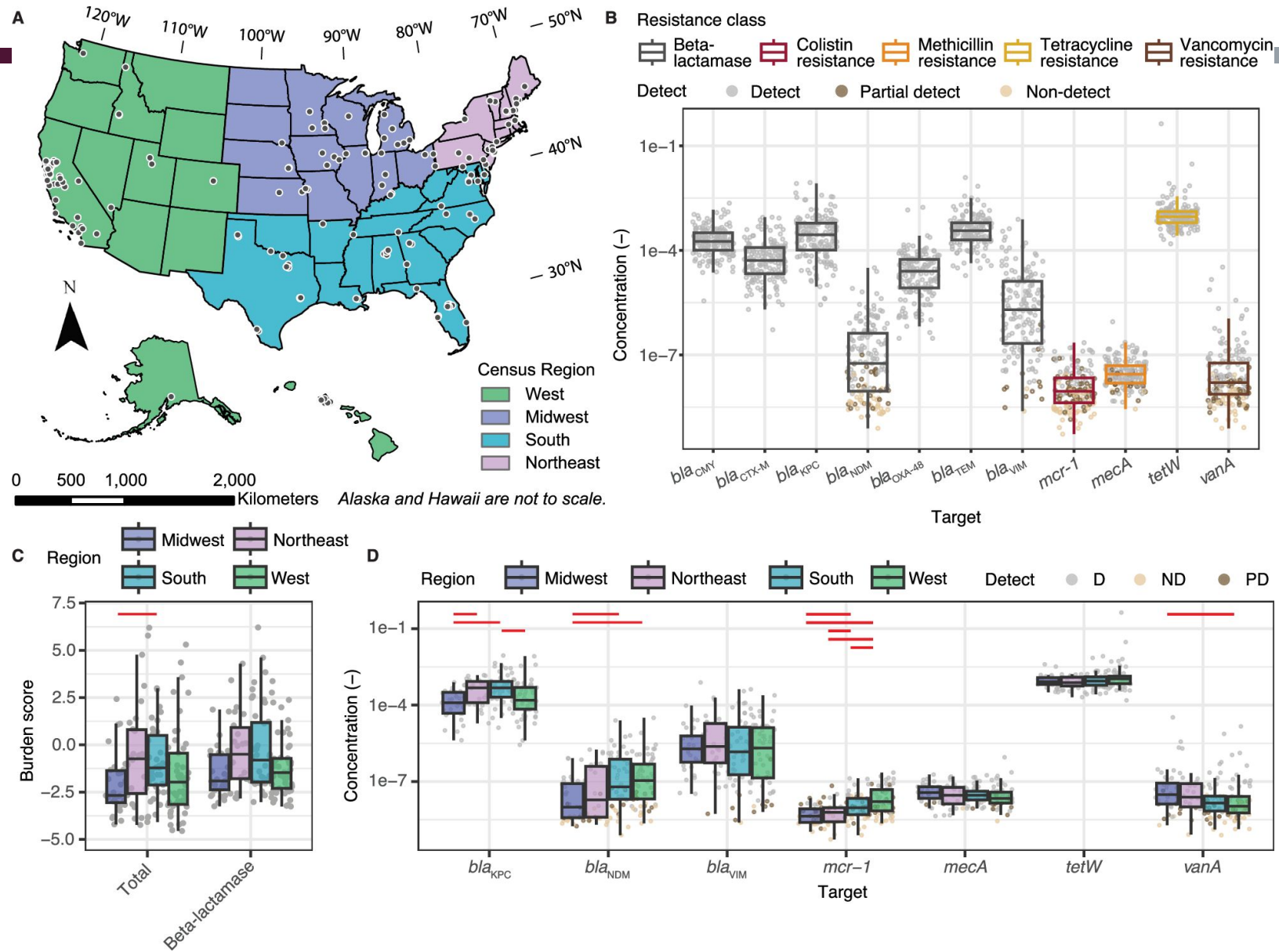
- **25 WWTPs | 5.2 million population | 2017–2019**
- Monitored **ESBL-E, CRE, VRE and MRSA** in untreated wastewater.
- **ESBL-E was consistently detected**, suggesting endemic circulation in the community.
- **CRE, VRE and MRSA were detected intermittently.**
- AMR patterns were associated with **different drivers**:
 - **CRE**: strongly linked to healthcare exposure/length of hospital stay.
 - **VRE**: associated with community factors, including school density.
 - **ESBL-E**: influenced by demographic, education and healthcare factors.



Rahman Z, Liu W, Stapleton L, Kenters N, Dewi DA, Gudes O, Ziochos H, Khan SJ, Power K, McLaws ML, Thomas T. Wastewater-based monitoring reveals geospatial-temporal trends for antibiotic-resistant pathogens in a large urban community. *Environmental Pollution*. 2023 May 15;325:121403.

NATIONAL WASTEWATER SURVEILLANCE OF AMR: U.S. STUDY

- **163 wastewater treatment plants | 40 U.S. states**
- **Quantified 11 antibiotic resistance genes (ARGs)** using digital droplet PCR.
- **Higher overall ARG concentrations in the South** compared with the Midwest.
- **Linked wastewater data with antibiotic use, social vulnerability, healthcare, animal agriculture and international travel.**
- **Social vulnerability and international travel** were associated with higher ARG concentrations.
- **Antibiotic use showed only a weak positive association**, highlighting the multifactorial nature of AMR spread.
- **Generated predictive maps of community ARG concentrations** and established a **national baseline**.



Kim, S., Zulli, A., Chan, E.M.G. *et al.* Wastewater surveillance reveals patterns of antibiotic resistance across the United States. *Nat Commun* 17, 4680 (2026).
<https://doi.org/10.1038/s41467-026-71195-4>

WASTEWATER-BASED AMR SURVEILLANCE IN HOSPITALS: GOALS

- **Identify AMR hotspots**
- **Enable timely IPC interventions**
- **Monitor outbreaks**
- **Support empirical treatment guidelines**
- **Understand hospital–community transmission**
 - When hospital WBS is combined with community wastewater surveillance, it may help investigate whether resistant organisms are primarily associated with the hospital or are also circulating in the surrounding community.
- **Benchmark AMR and antibiotic consumption**
- **Provide early warning of emerging threats**
 - Detect emerging **multidrug-resistant organisms (MDROs)** or specific resistant clones.
 - Potentially identify emerging strains before they become widespread.

MONITORING AMR UNDER THE URBAN WASTEWATER TREATMENT DIRECTIVE (UWWTD) EUROPE

Regulatory Mandate

- The recast **Urban Wastewater Treatment Directive (UWWTD)** requires AMR monitoring in large European agglomerations.

Harmonization Framework (EU-WISH & EU-JAMRAI 2)

- EU-WISH:** Focuses on strengthening public health wastewater surveillance platforms.
- EU-JAMRAI 2:** Develops environmental surveillance approaches for AMR.

Current Implementation Landscape (2024 EU-WISH Survey)

- Uptake:** Only **11 of 27 surveyed countries** currently operate an active AMR WBS system.
- Primary Objective:** Track broad community AMR trends.
- Current Methodologies:** Culture-based assays, strain-specific characterization, and qPCR for targeted resistance genes (metagenomics used sparingly).

Strategic Roadmap for Target Selection

- Align Objectives:** Define whether surveillance targets human AMR trends, emerging resistance determinants, environmental release, or wastewater treatment efficiency.
- Prioritization Criteria:** Select indicators based on **public health relevance**, practical **measurability**, and **resource efficiency**.
- One Health Integration:** Combine cross-sectoral expertise across human, animal, and environmental health domains.

TABLE

General overview of ideal characteristics of targets and methods to fulfil specific objectives of AMR surveillance at community wastewater treatment plants, 2024

Target and methods characteristics		Objectives of monitoring				
		WWTP influent		WWTP effluent		WWTP efficiency
		(i) AMR trends in community	(ii) Early detection of emerging AMR	(iii) AMR emissions ^a	(iv) Antibiotic residue emissions ^a	(v) AMR reduction efficiency
Targets	Human public health relevant	Relevant	Relevant	Relevant	Relevant	LR
	Animal health relevant	Relevant	Relevant	Relevant	Relevant	LR
	Environmental health relevant	LR	LR	Relevant	Relevant	LR
	Regularly detectable	LR	LR	Relevant	Relevant	Relevant
Methods	Culture-based	Relevant	Relevant	Relevant	LR	Relevant
	PCR-based	Relevant	LR	Relevant	LR	Relevant
	Metagenomic sequencing	Relevant	Relevant	Relevant	LR	LR
	LC-MS	LR	LR	LR	Relevant	LR

AMR: antimicrobial resistance; LC-MS: liquid chromatography mass spectrometry; LR: less relevant; WWTP: wastewater treatment plant.

^a Emissions via WWTP effluent to surface water.

Relevant targets are in bold font.



Metagenomics has great potential for community-level AMR surveillance
Current approaches focus on pathogens isolated from symptomatic infections

Sample collection



Representative sampling:

- AMR load
- Captured demographic
- Loss of quality in processing
- Bias from enrichment
- Source contamination

DNA extraction



Extraction quality and accuracy:

- Method variability
- Side-effects of extraction on DNA
- Need for reference reagents

Sequencing



Appropriate sequencing approach:

- Resistome versus taxonomic focus
- Long-read, short-read, and others
- Need for reference reagents

Bioinformatics

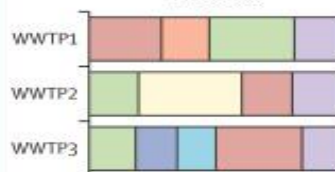


Appropriate data analysis:

- Resistome versus taxonomic focus
- Long-read, short-read, and others
- Need for reference reagents

Methodological choices, from sample collection to data analysis, impact data quality, and ultimately, the interpretation of results

Method 1



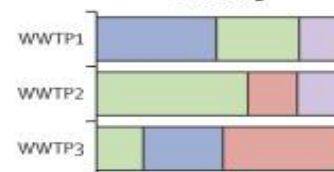
Relative abundance

Method 2



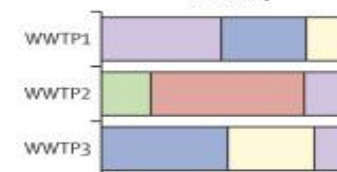
Relative abundance

Method 3



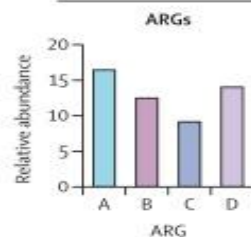
Relative abundance

Method 4



Relative abundance

As a result of varying methodologies, the same sample can produce differing results



Genotype-phenotype
Biofilms
Tolerance
Community interactions

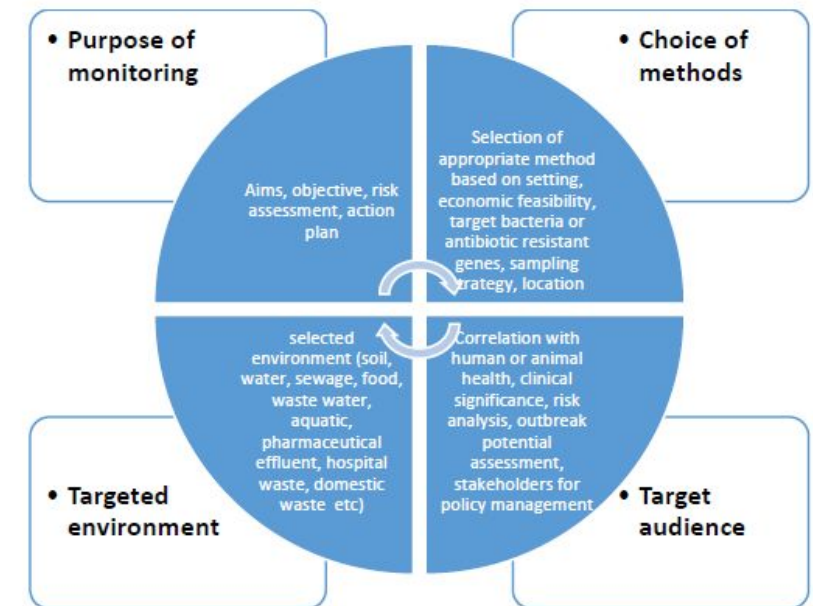
Potential for metagenomic AMR surveillance to inform public health policy and antimicrobial stewardship
A need for standardisation of methods and addressing other determinants of susceptibility phenotype

COMMUNITY BASED SURVEILLANCE FOR AMR MONITORING: REQUIREMENTS IN LMICS

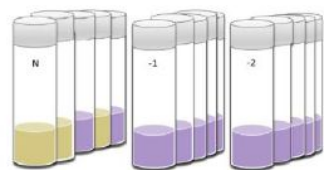
Figure 1
System requirements to establish community based amr surveillance program in LMICs



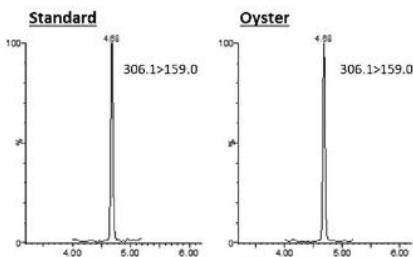
Figure 2
Technical Requirements to Establish Community Based AMR Surveillance in LMICs



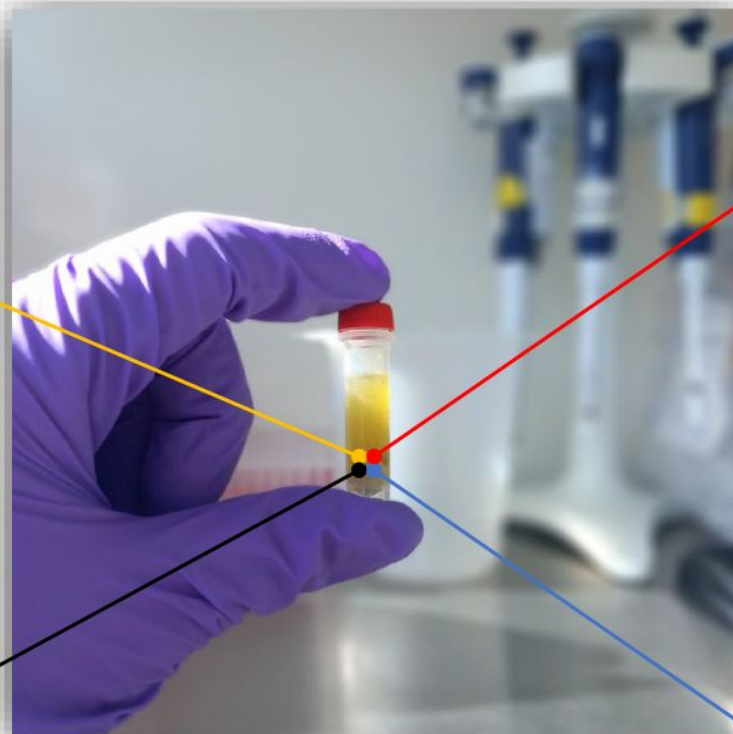
"One Sample Many Analyses" (OSMA)



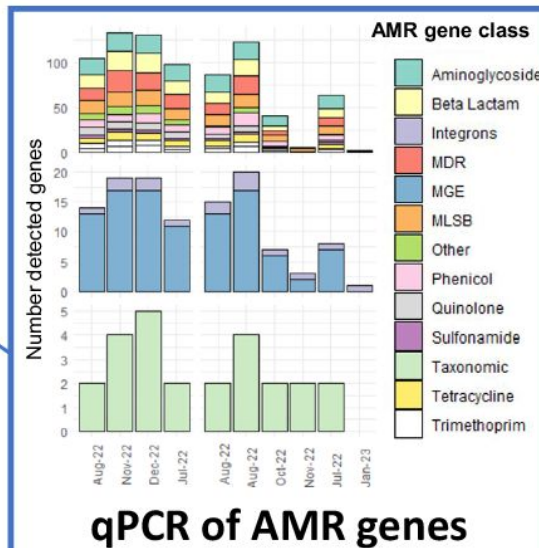
AMR analysis



Chemical residue screening



16S and metagenomic analysis



FROM SURVEILLANCE TO ACTION

- Wastewater is not simply a waste stream.
- It is a window into population health and AMR circulation.
- The future of AMR surveillance is not wastewater alone—it is wastewater integrated with clinical, epidemiological, genomic and One Health intelligence.



THANK YOU

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