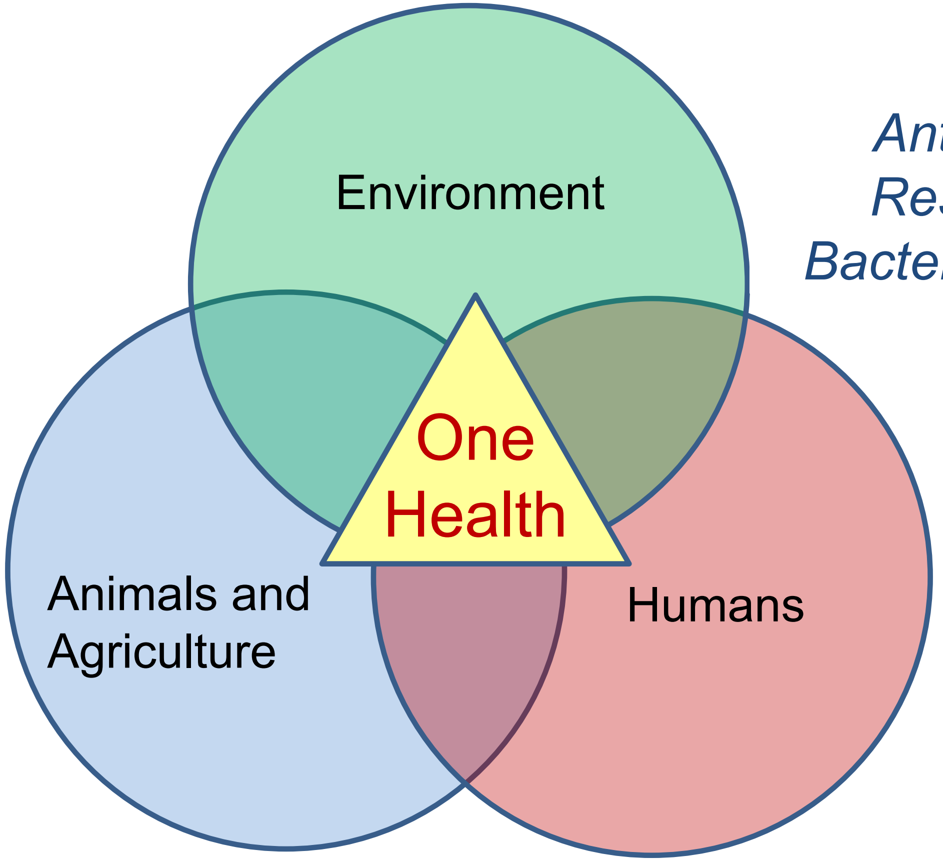
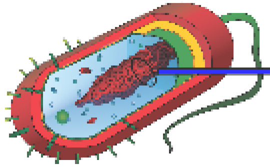
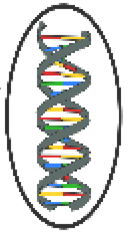
# Informing Policy & Practice for Combating AMR Through Surveillance of Water and Wastewater Environments

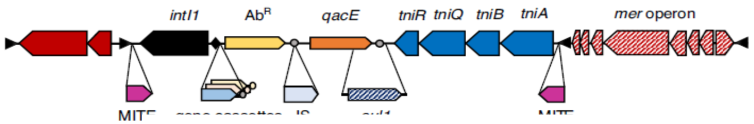


*Antibiotic Resistant Bacteria (ARB)*



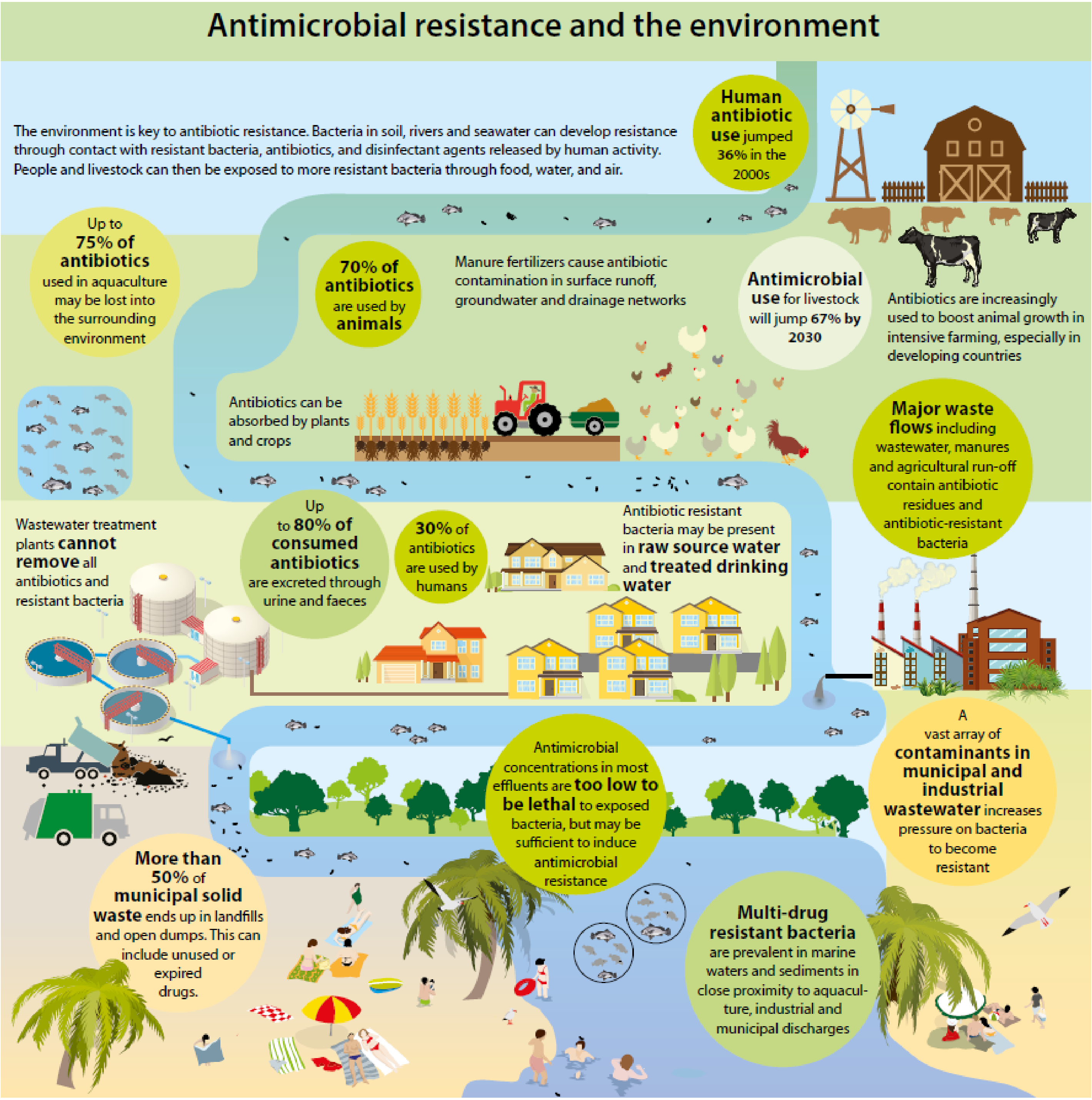
*Antibiotic Resistance Genes (ARGs)*




*Mobile Genetic Elements (MGEs)*

Amy Pruden
University Distinguished Professor
*Via Department of Civil & Environmental Engineering*
VIRGINIA TECH

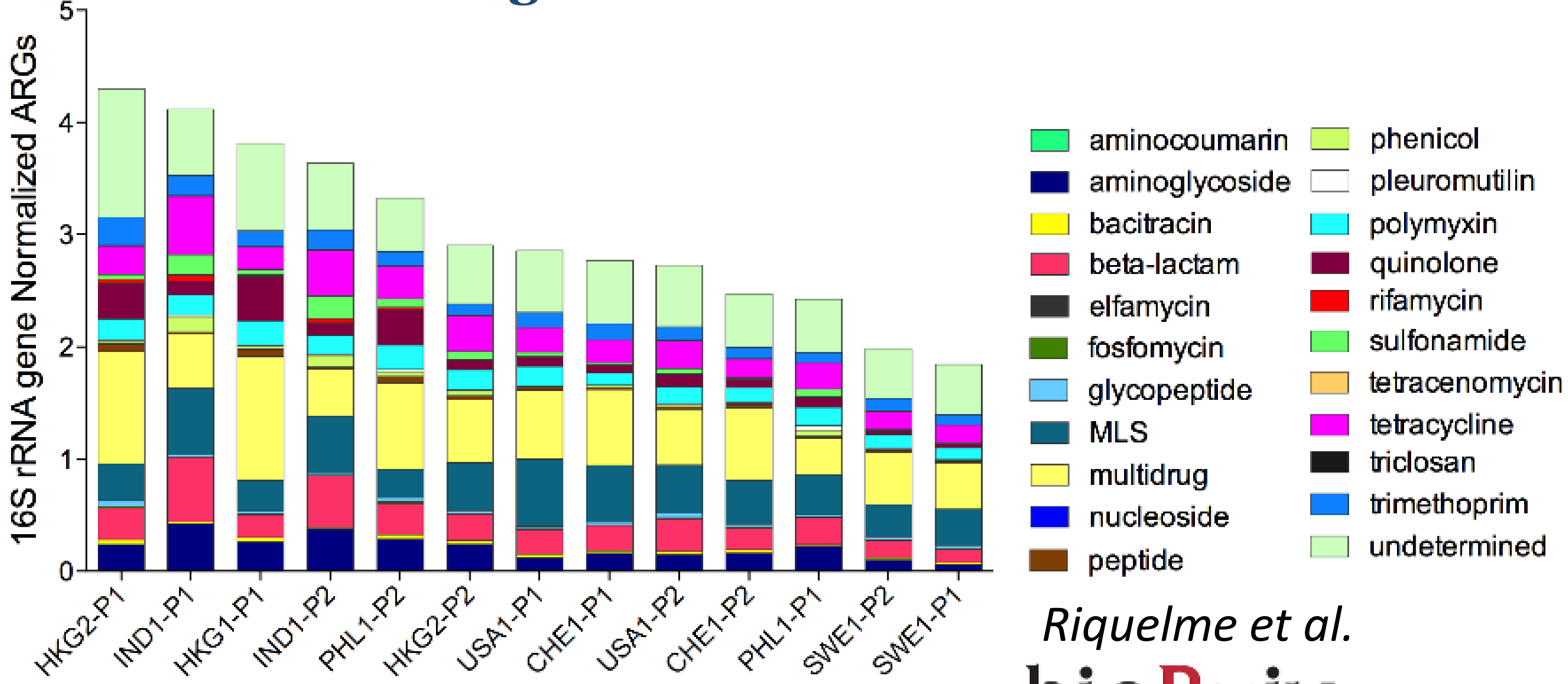
# The Environment: The missing piece to **One Health** action to combat AMR




UN environment

# Global Metagenomic ARG Survey (*Presented at PACCARB Oct 2018*)

## INFLUENT Sewage: Ranked "Total ARG" Abundance




**Locations:**
CHE – Switzerland
HKG – Hong Kong
IND – India
PHL - Philippines
SWE – Sweden
USA – United States

*Riquelme et al.*
bioRχiv

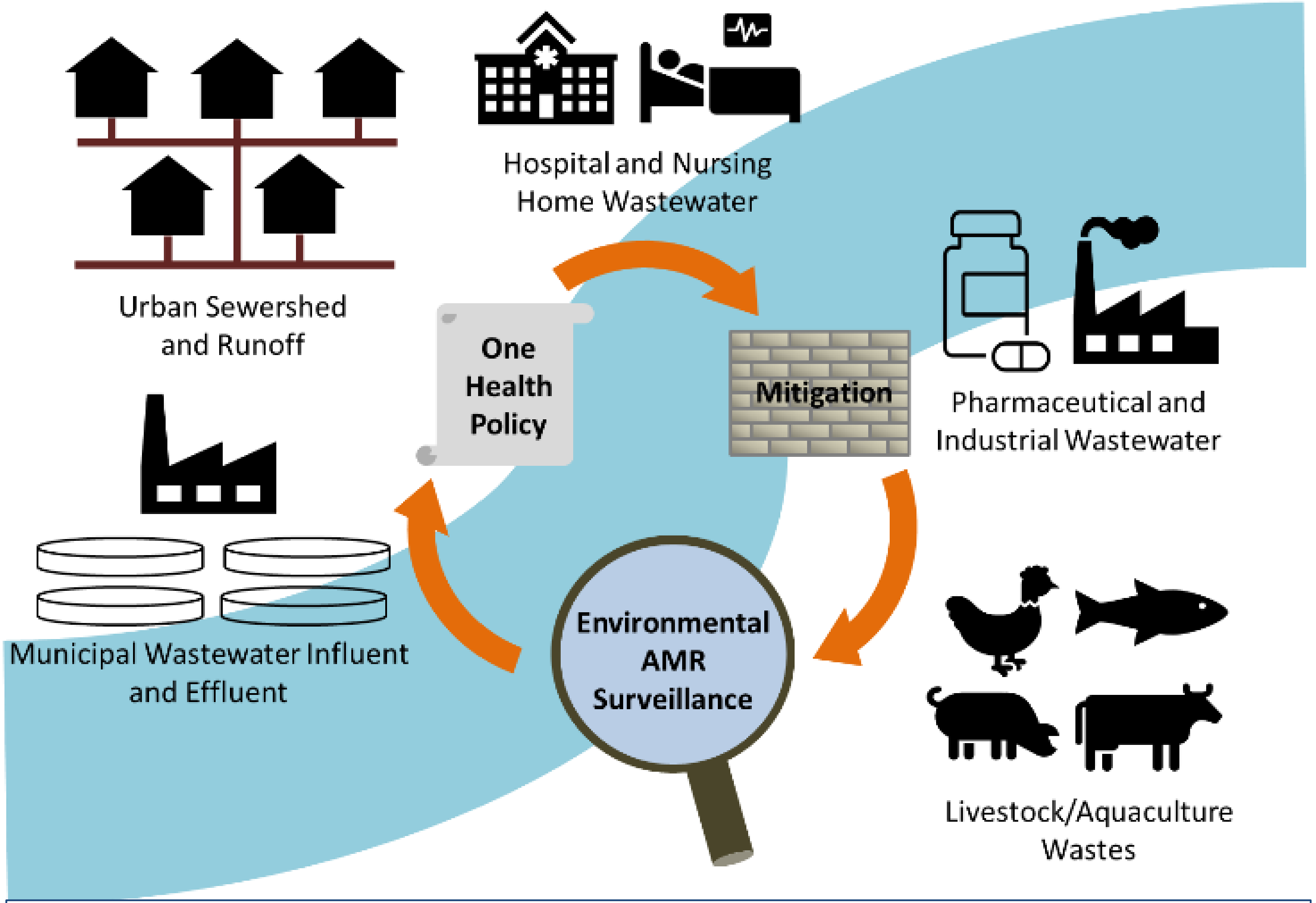
-Highest in Hong Kong and India
-Lowest in Sweden

CARD
Use or Download Copyright & Disclaimer

*Partnership in International Research and Education PI: Peter Vikesland, VTech*

# Integrated Environmental AMR Surveillance to Inform Solutions

- Wastewater and surface water can provide integrated One Health surveillance points to inform solutions
- Provide large, comparable longitudinal datasets for identifying drivers of AMR and predicting trends
- Identify epidemiological links between the environment, humans, and animals
- Providing data needed to inform risk assessment models and target regulatory limits
- Identify hotspots for evolution and spread of AMR
- Identify treatment technologies that most effectively mitigate AMR spread
- Inform human and animal medicine regarding which antibiotics will be most effective at population-specific scales




*Data-informed policy to guide investment of resources to combat AMR*

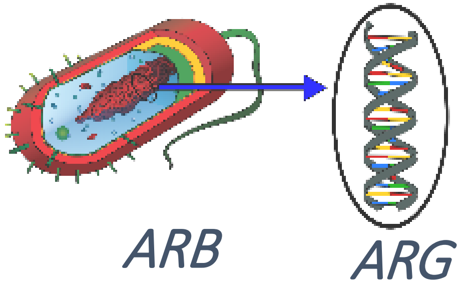
Pruden et al. *Curr. Opin. Microbiol.* in Review

# Growing Interest in Environmental Surveillance of AMR: US Water Utilities and Public Health Agencies

- Water Research Foundation Projects:
  - ***Project 4813:*** *Critical Evaluation and Assessment of Health and Environmental Risks from Antibiotic Resistance in Reuse and Wastewater Applications*
  - ***Project 4961:*** *The Use of Next Generation Sequencing (NGS) Technologies and Metagenomics Approaches to Evaluate Water and Wastewater Quality Monitoring and Treatment Technologies*
  - ***Project 5052:*** *Standardizing Methods with QA/QC Standards for Investigating the Occurrence and Removal of Antibiotic Resistant Bacteria/Antibiotic Resistance Genes (ARB/ARGs) in Surface Water, Wastewater, and Recycled Water (Expert Workshop Held May 2021)*

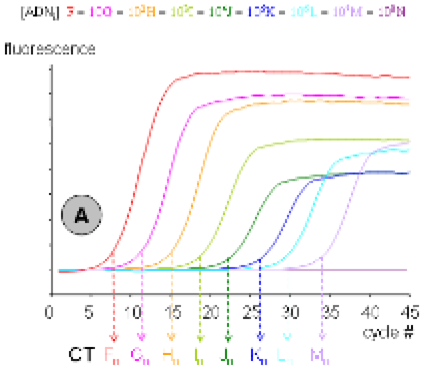
THE Water Research FOUNDATION

# Culture/ Genomics


- Enumerate resistant pathogens/ indicators of interest
- Can whole genome sequence isolates for source tracking
- Can assay multi-AMR via phenotypic or genotypic testing
- Directly inform risk assessment

# qPCR




- Precise quantification of specific ARGs/MGEs across microbial community
- Circumvents culture bias
- Quantitative data useful for risk and other modeling

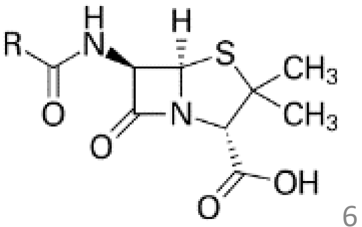
# Metagenomics

Wikimedia Commons

- Broad profiling of ARGs/MGEs across microbial community (“resistome”)
- No need to select targets *a priori*

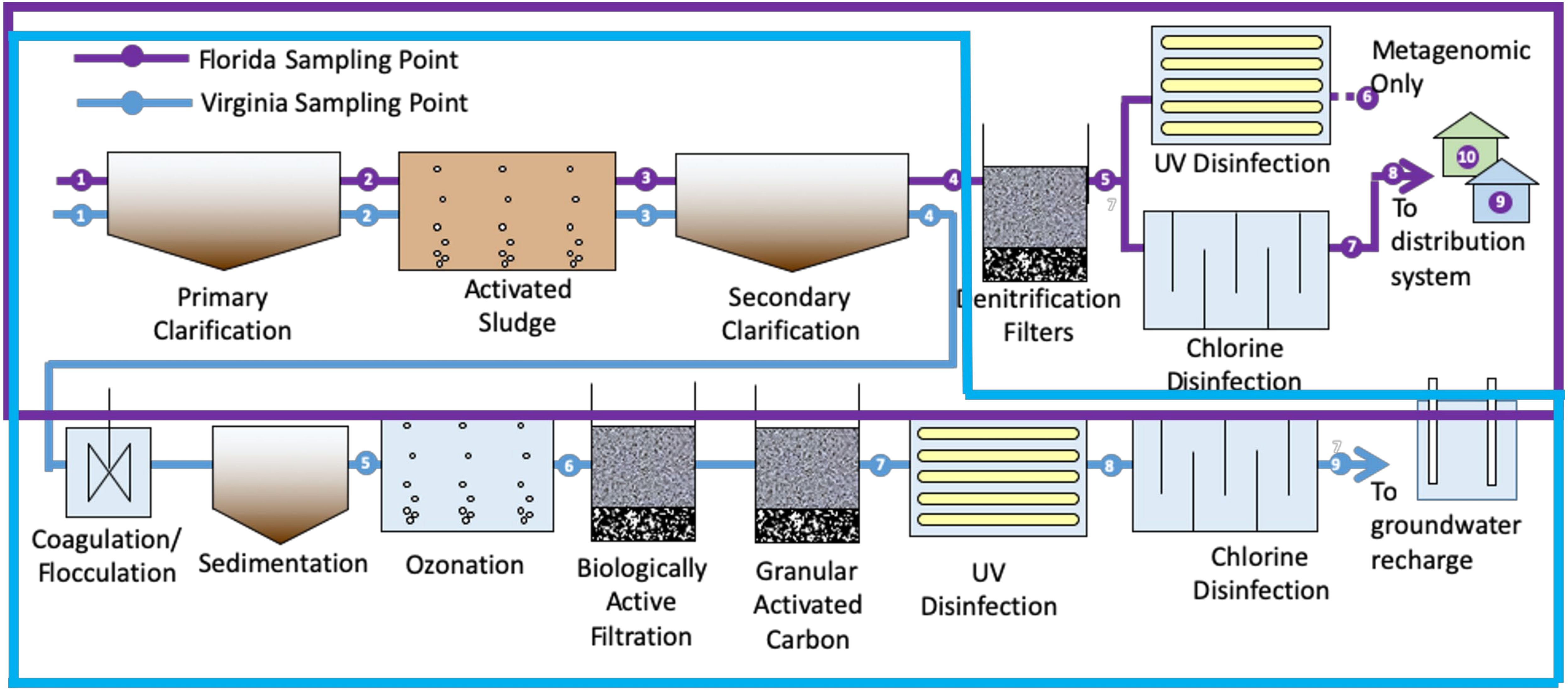
## Antibiotic Monitoring (target or non-target analysis)

- Which antibiotics being used in a population
- Which degrade during treatment
- *Helps circumvent poor reporting of antibiotic use*



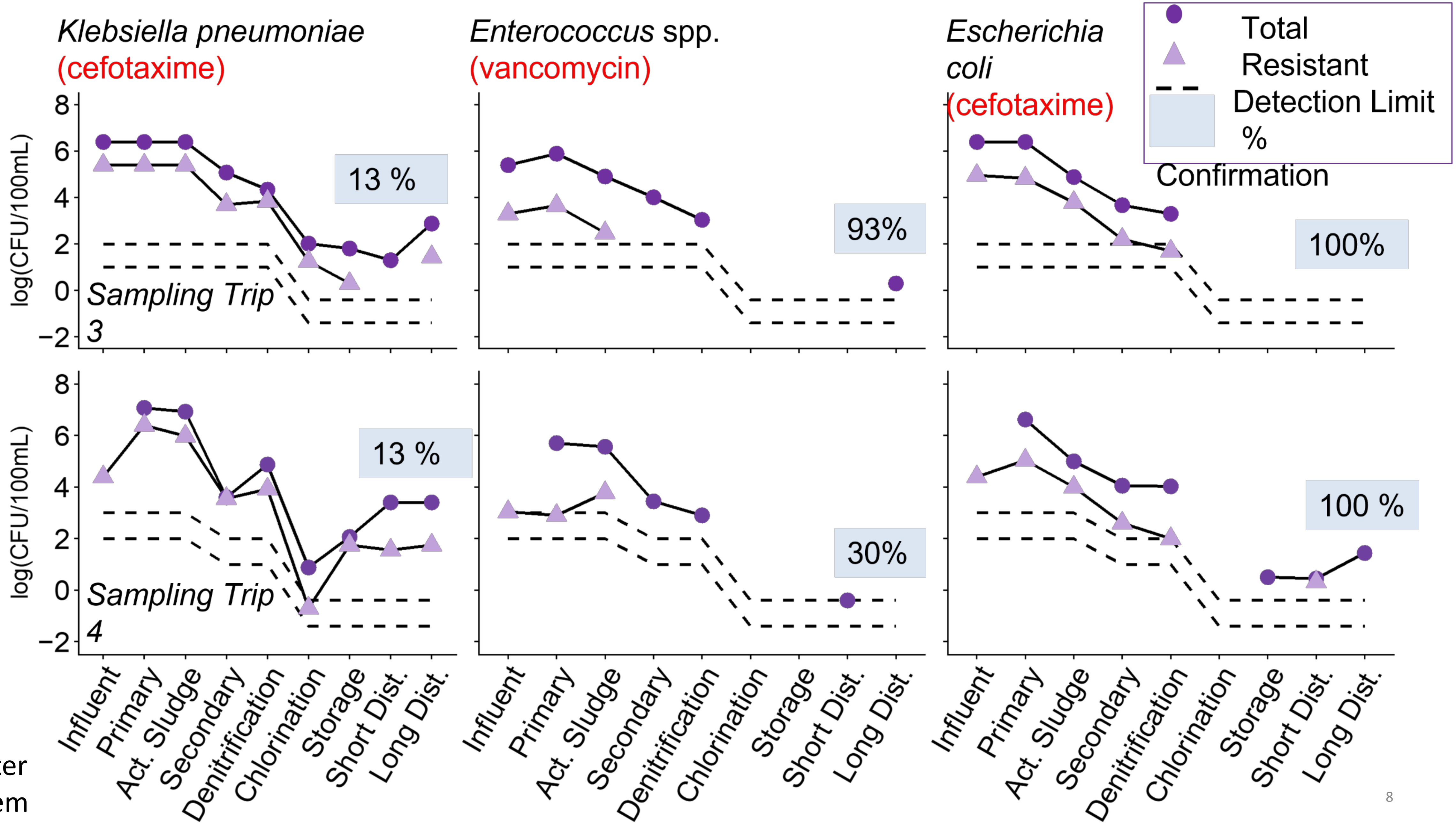

Penicillin- Wikimedia Commons

# Tracking ARGs and Pathogens in Wastewater and Water Reuse Systems



# Tracking ARBs in Wastewater and Water Reuse Systems

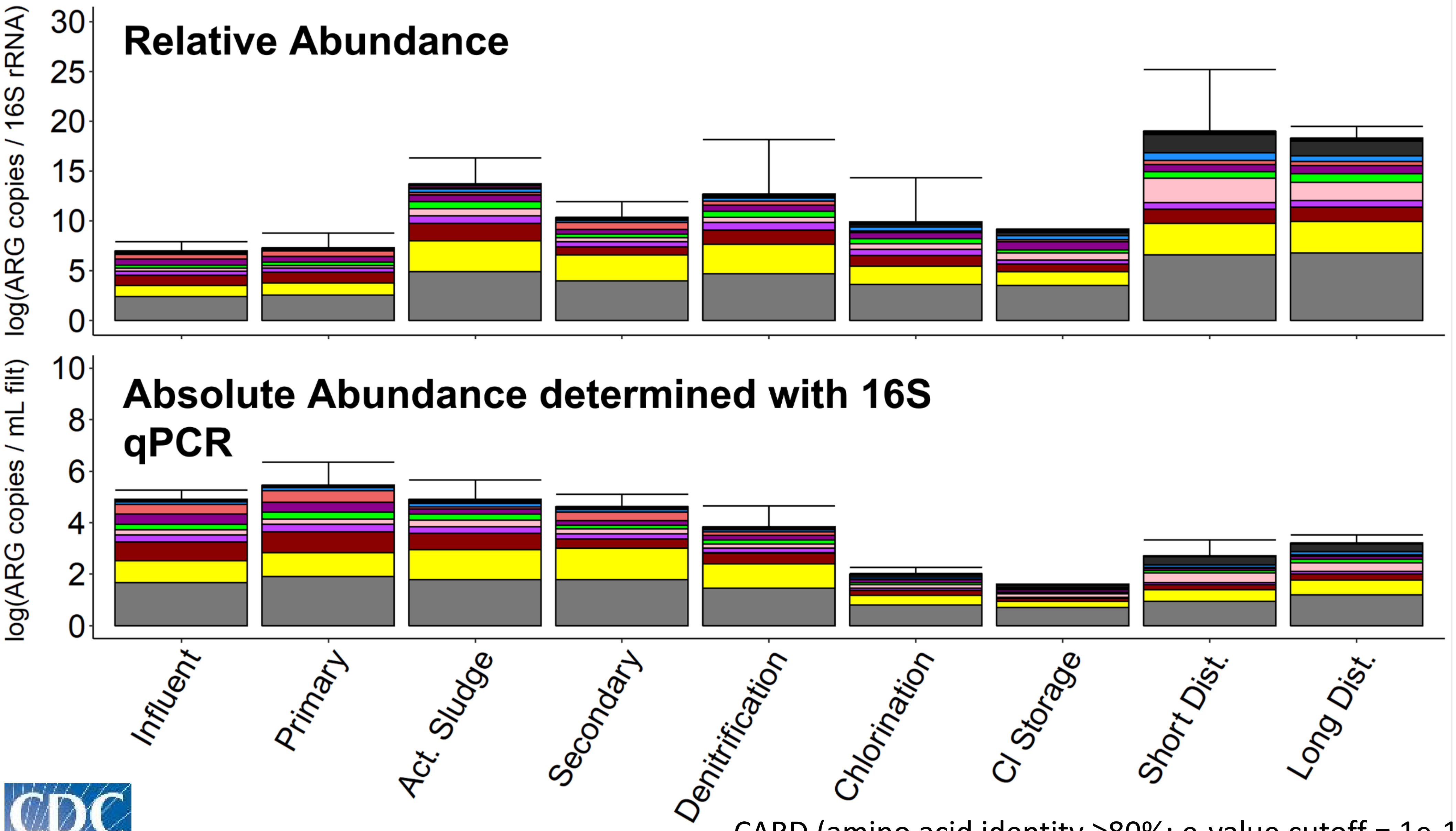
*Jody Harwood, Co-PI, U. S. Florida*




CDC
Centers for Disease Control and Prevention

Florida Water Reuse System

# Tracking Resistomes in Wastewater and Water Reuse Systems



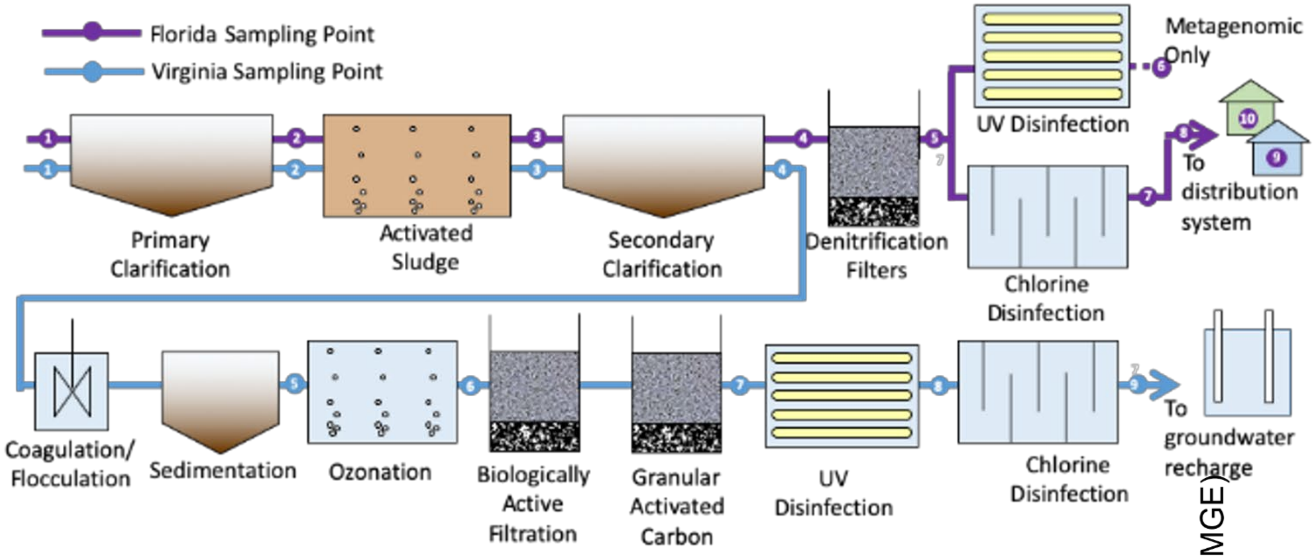

Ishi Keenum
PhD Student

Florida Water Reuse System

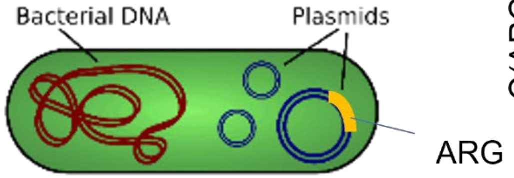
CARD (amino acid identity ≥80%; e-value cutoff = 1e-10; minimum alignment length = 37 amino acids)

# Shifts in "Resistome Risk" through Wastewater and Reuse Treatment

*Liqing Zhang, Co-PI, VT*



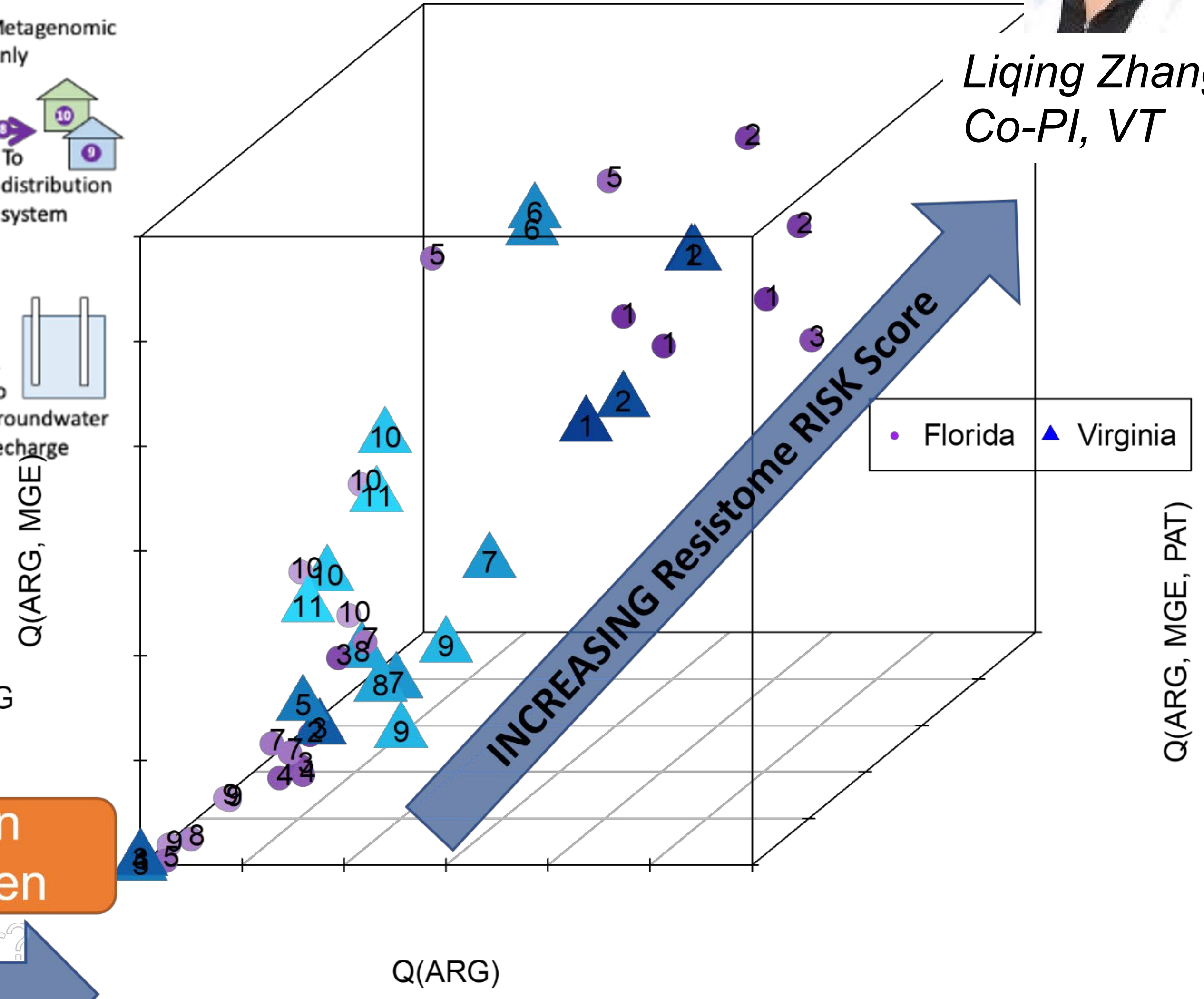

## MetaCompare Pipeline:




Wikimedia Commons

Assembled Contig or long read → ARG? → Yes? → Mobile Genetic Element? → Yes? → Human Pathogen Marker?

**INCREASING Resistome RISK Score**




*Numbers refer to stage of treatment and distribution*

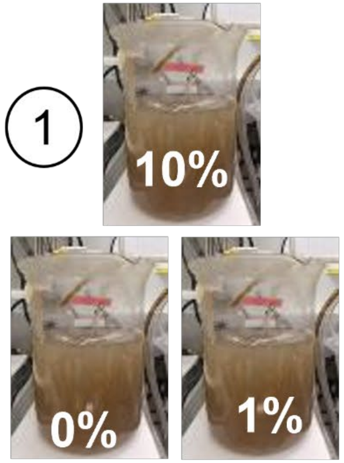
Oh et al. 2018. *FEMS Microbiol. Ecol.*

# Lab-Scale WWTPs:
## *Should Hospital Wastewater be Treated On-Site?*

Connor Brown
PhD Student

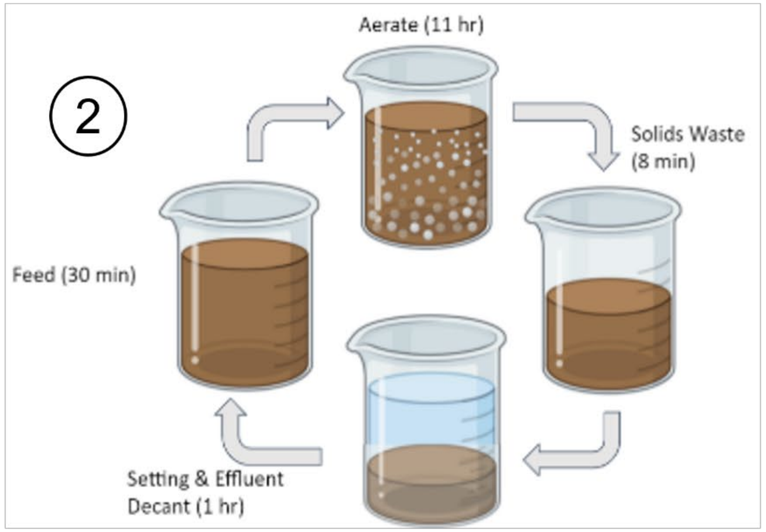
Ayella Maile-Moskowitz
PhD Student

1




**Various concentrations of hospital sewage**

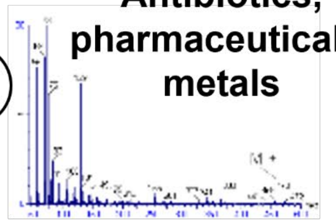
2



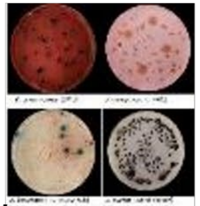

**Sequencing Batch Reactors**

3

**Antibiotics, pharmaceuticals, metals**



**Culture ARBs**



**Illumina Sequencing**

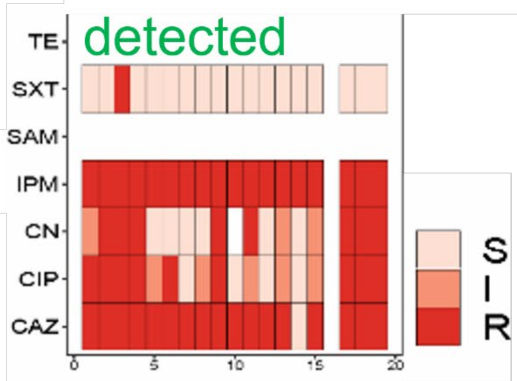
**Long-Read Sequencing**

*Klebsiella pneumoniae*, *Escherichia coli*, Carbapenemase-producing ARBs

hospital sewage

0% → No resistant *Aeromonas* detected

10% →




ChroMagar mSuperCARBA

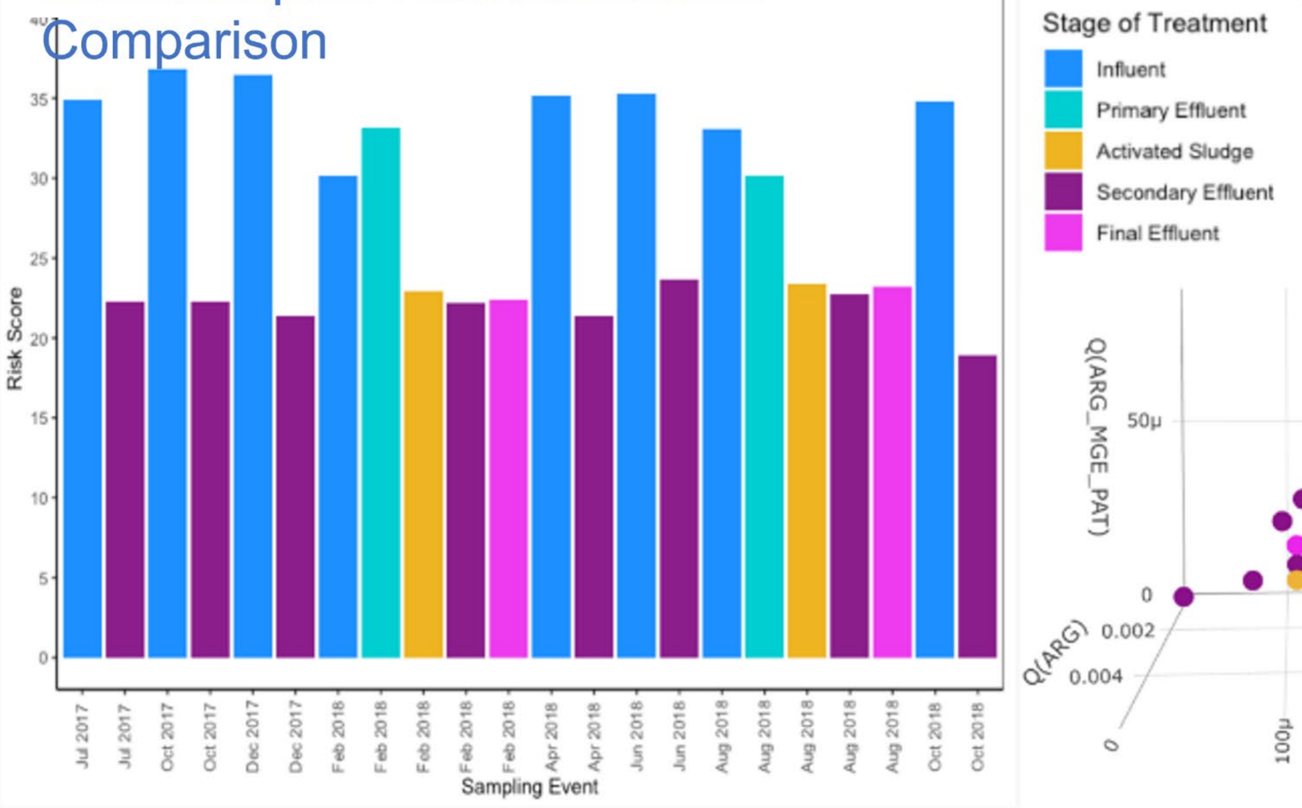
- Isolated >450 antibiotic resistant bacteria (*Klebsiella pneumoniae, Escherichia coli,* and Carbapenemase producing organisms)
- Sequenced ~6x10$^{11}$ basepairs of Illumina and Nanopore-derived sequences across 114 samples
- Non-target antibiotic analysis of 27 samples

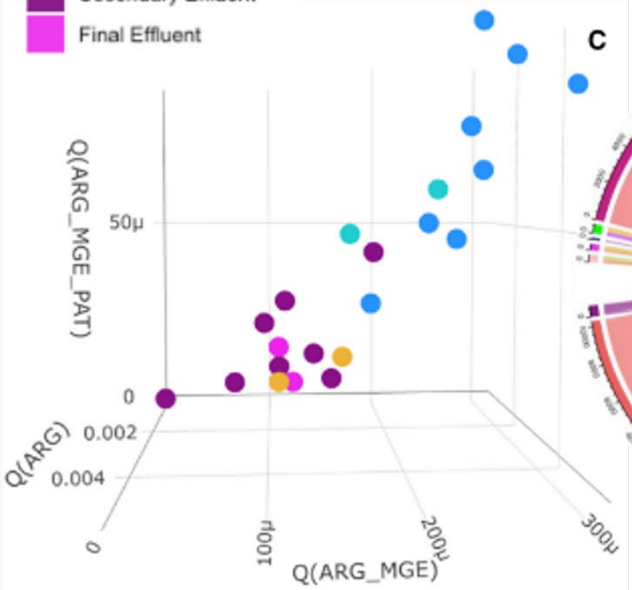
# Effluent is a Clinically- and Treatment- Relevant Monitoring Point

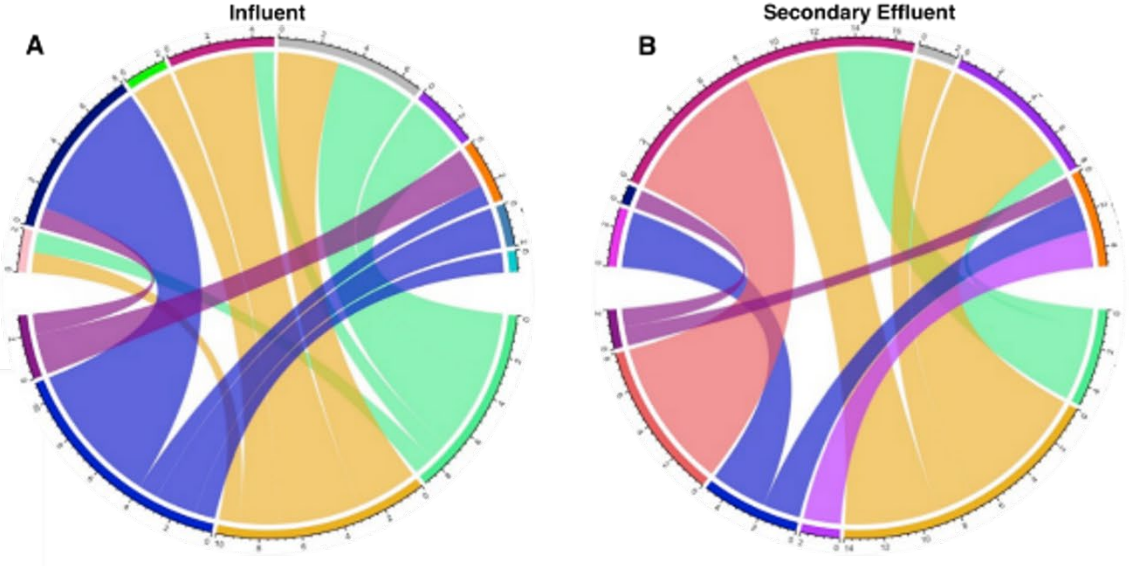
- 18-month longitudinal metagenomic analysis through a local WWTP

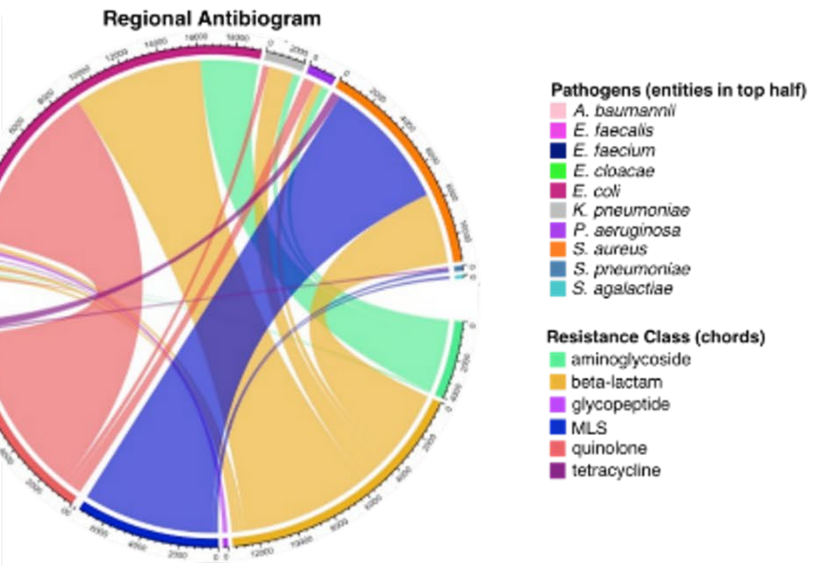
Haniyyah Majeed et al. *Frontiers in Microbiology* 2021

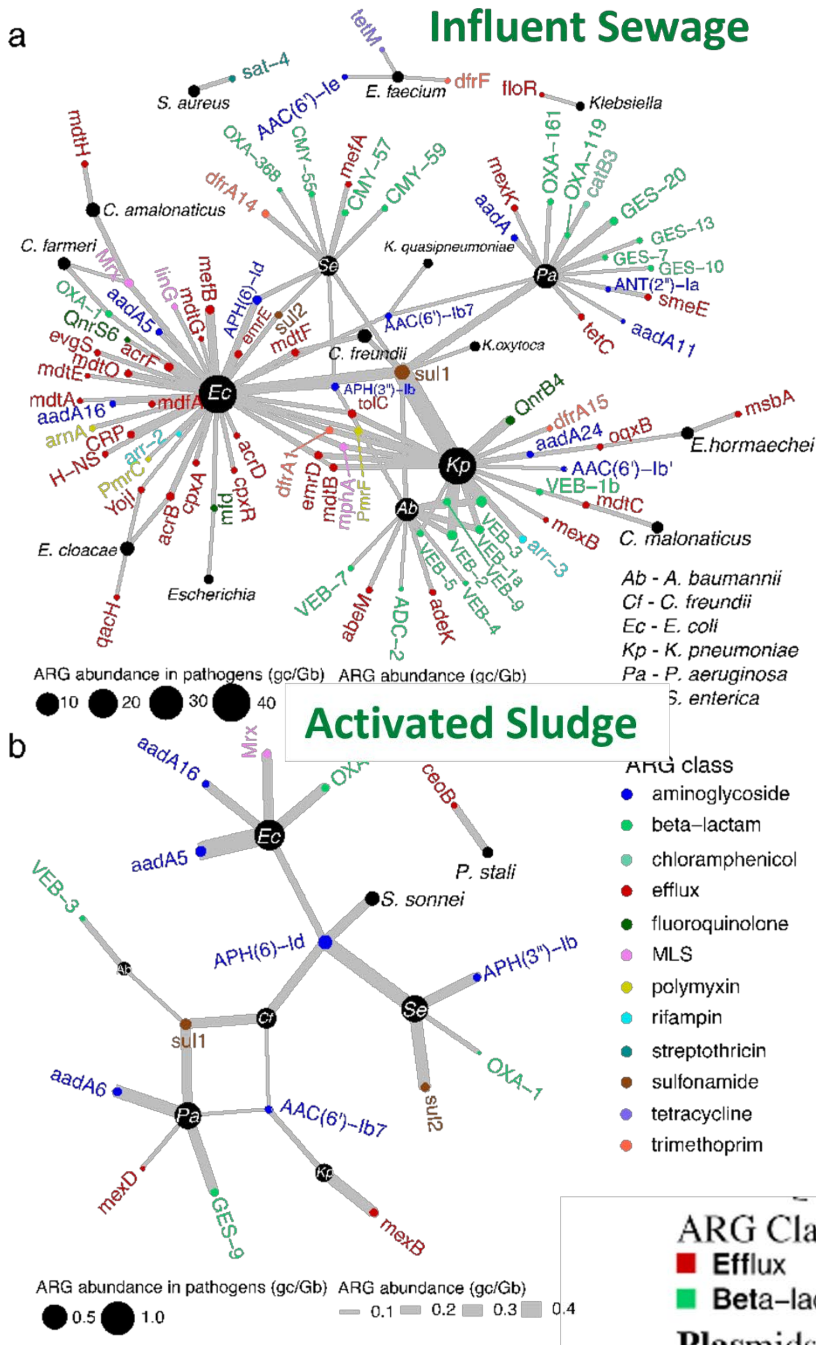
## MetaCompare Resistome Risk Comparison



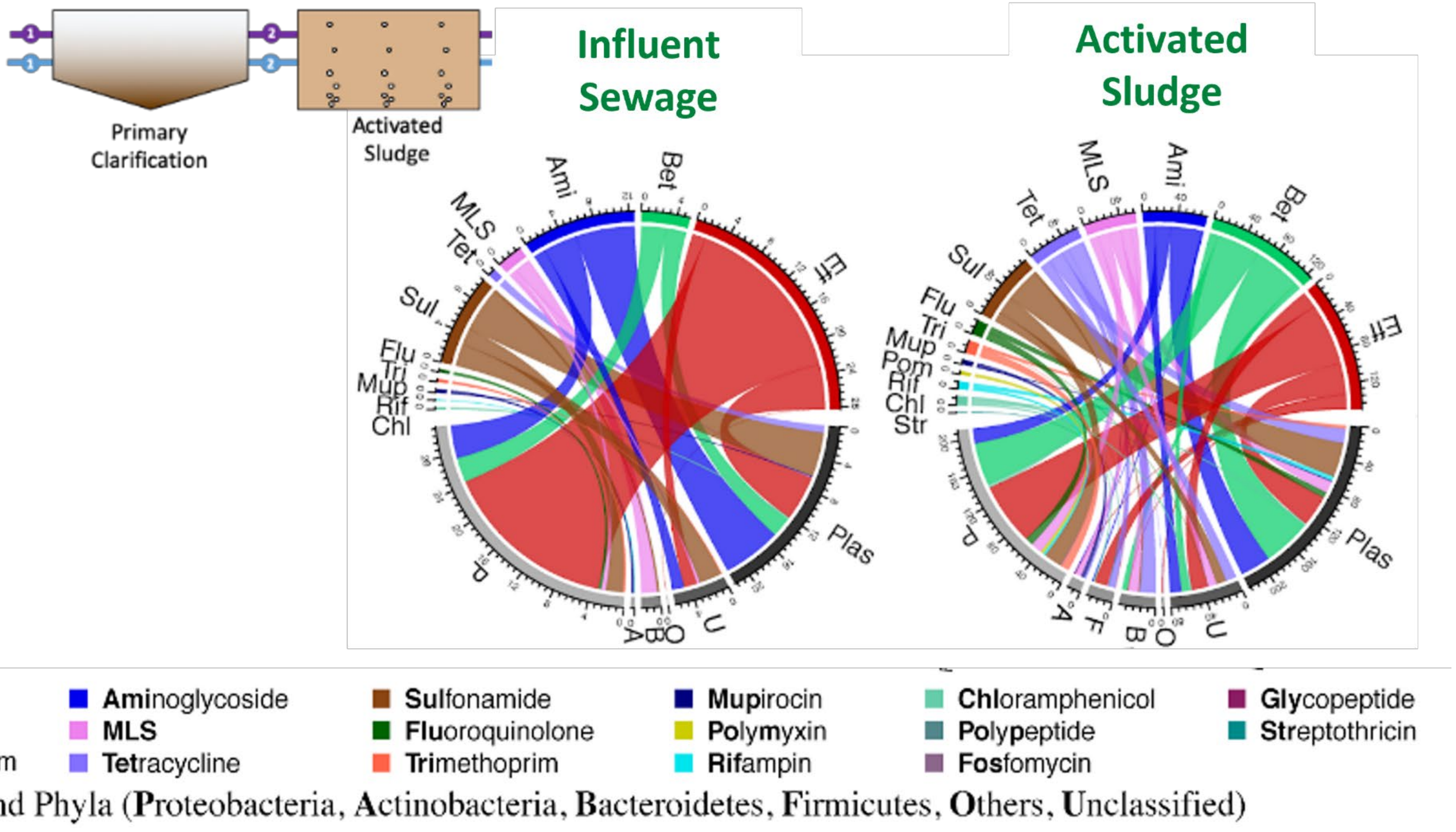





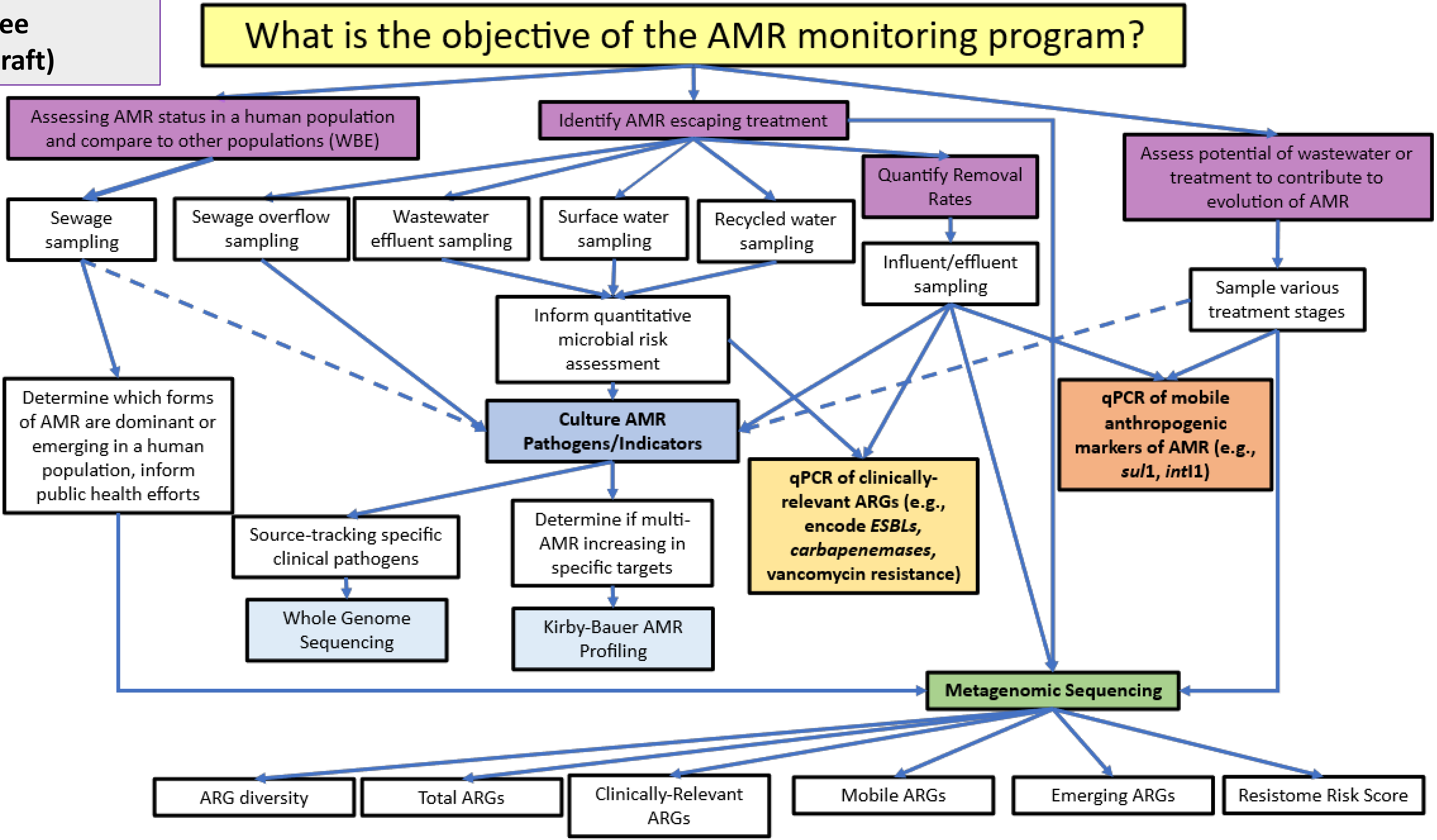

# Benefits of Long-Read Metagenomic Sequencing

Dongjuan Dai et al. *In Review*

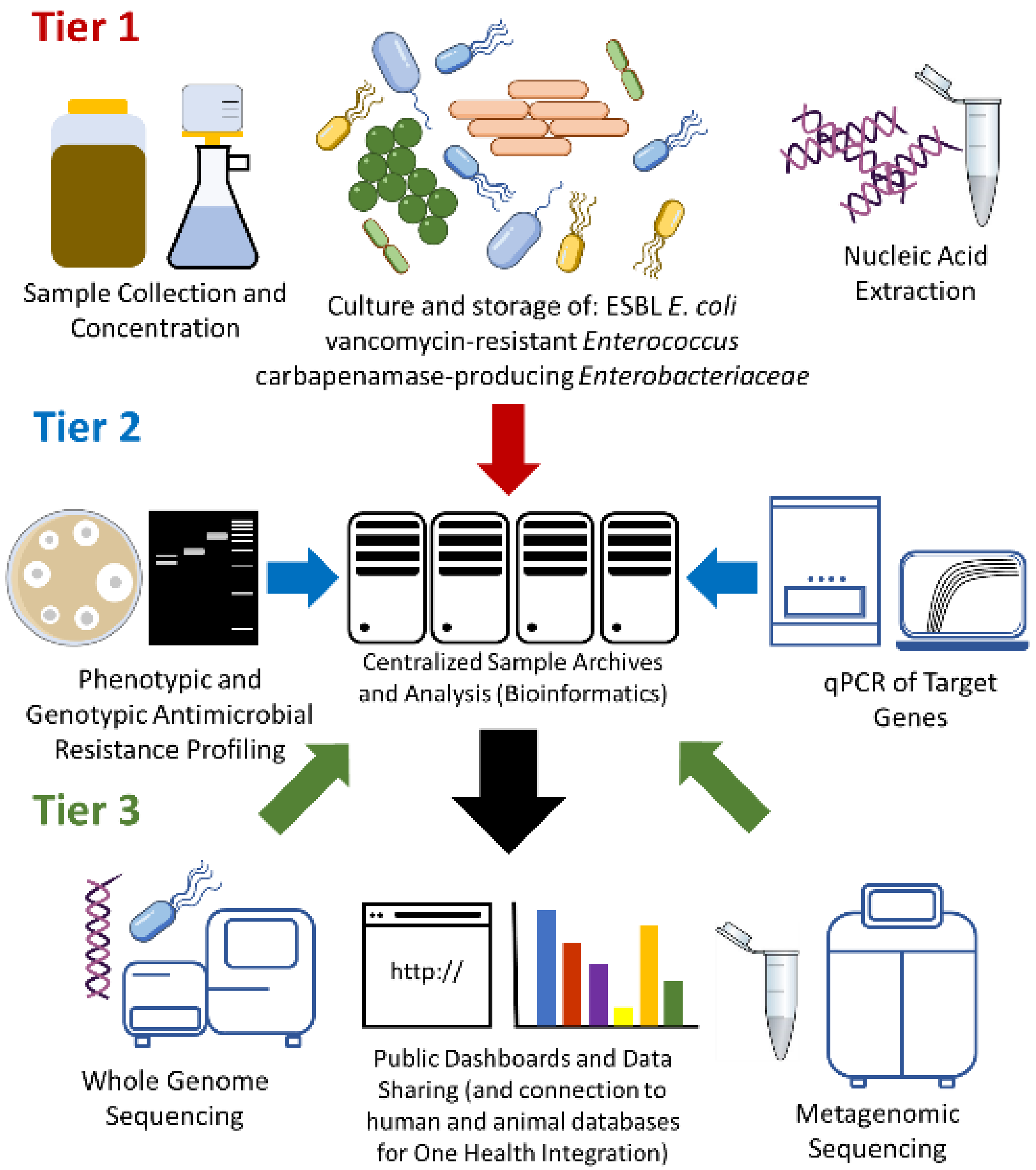
- *Who* is Carrying the ARGs, are they potentially pathogenic?
- Evidence that ARGs carried on MGEs



**WRF Project 5052 Decision-Tree (Working Draft)**



# Tiered Approach to Integrated Environmental AMR Surveillance




**Existing AMR Surveillance Systems**

- NARMS (US)
- NARMS Surface Water AMR Monitoring (SWAM) (US)
- EARS-net (Europe)
- EARS-vet (Europe)
- Global Sewage Surveillance Project (Denmark)
- Global Antimicrobial Resistance Surveillance System (GLASS) (WHO)
- **JPIAMR Tricycle Program (WHO) (US not yet a member!)**

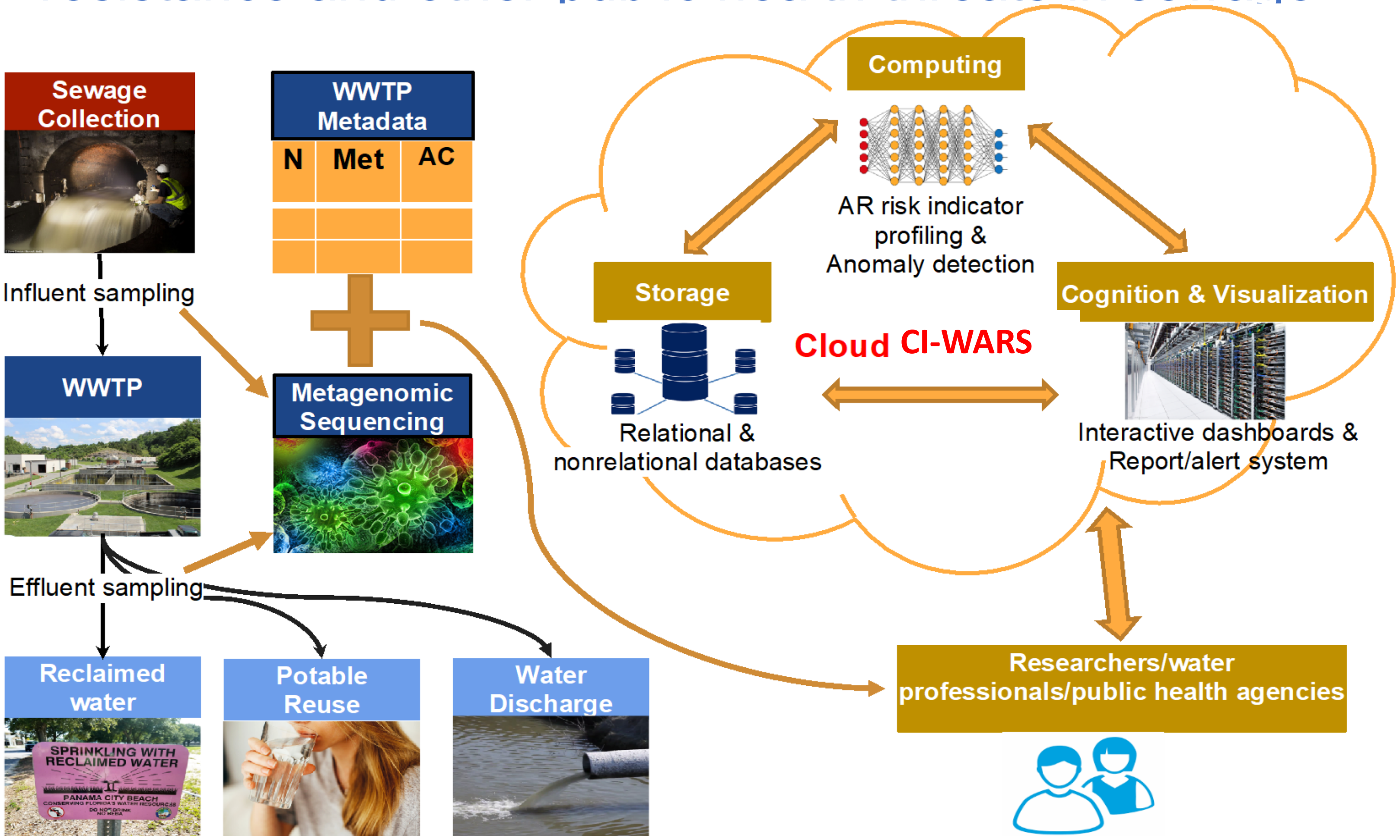
**What is Missing?**

- Full integration of human, animal, and environmental AMR monitoring data
- Standardized methods for culture- and molecular-based AMR monitoring
- Integration of multiple types of AMR monitoring data and metadata
- Centralized data accessibility and transparency with robust and informative metadata
- Risk assessment frameworks appropriate for environmental AMR and adaptable to different data types and questions

Pruden et al. *Curr. Opin. Microbiol*. in Review

# NSF Cyberinfrastructure for Sustained Scientific Innovation (CSSI) Award ($1.3M, 2020-2023)

## -CI-WARS- (“sewers”) Establishing cyberinfrastructure for monitoring antibiotic resistance and other public health threats in sewage




PI: Liqing Zhang

Co-PI: Amy Pruden

Co-PI: Peter Vikesland

Co-PI: Ali Butt


USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

Lenny Heath, Co-PI VT

http://agroseek.cs.vt.edu/

1) Crowd-sourcing to support computational and predictive data analysis
2) Collection of comprehensive metadata (e.g., livestock type, antibiotics used, manure management practices, crops grown, water plant configuration, water chemistry, DNA extraction kit, sequencing platform/configuration)
3) Compare with publicly-available data (e.g., are your metagenomic metrics high or low?)
4) Computational Modeling to identify critical control points for AMR in environmental systems and target with appropriate agricultural practices

# Building on Momentum in Wastewater Surveillance Public Dashboards: COVID-19

# Acknowledgements


CENTERS FOR DISEASE CONTROL AND PREVENTION

Contract # 75D35118C02904

USDA NIFA

United States Department of Agriculture
National Institute of Food and Agriculture

NSF

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