

# Detection and Control of Antibiotics and Antibiotic Resistance in the Environment:

## *Assessment and Analysis*

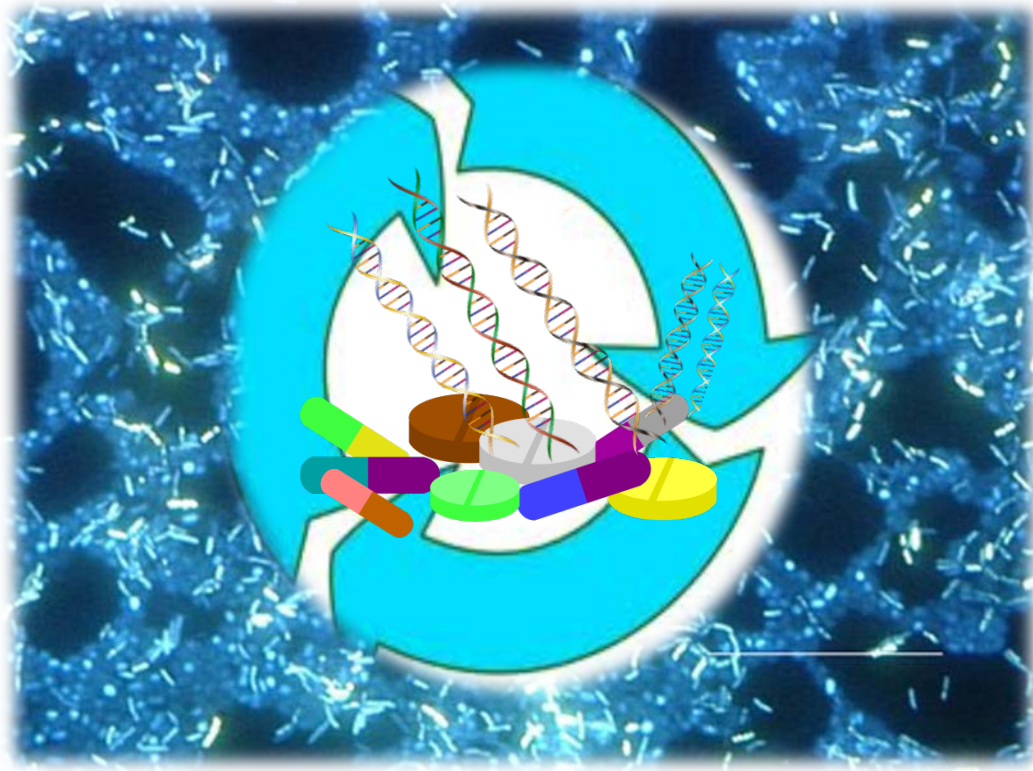


Image: Rodney M. Donlan, CDC

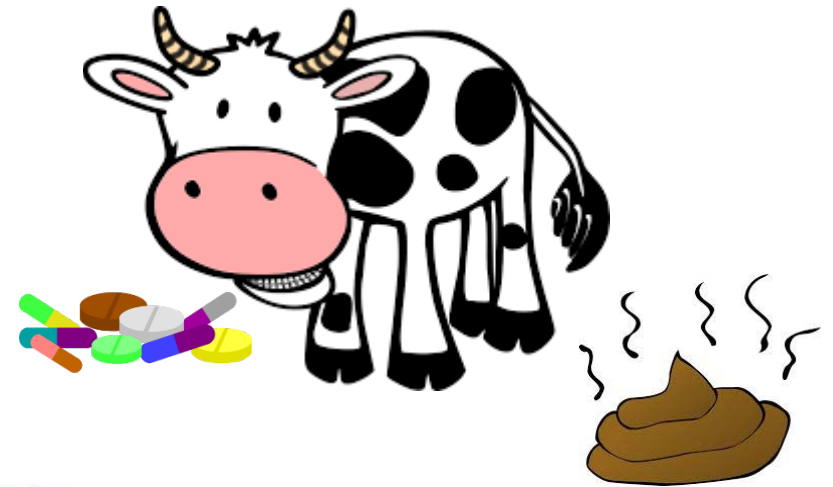
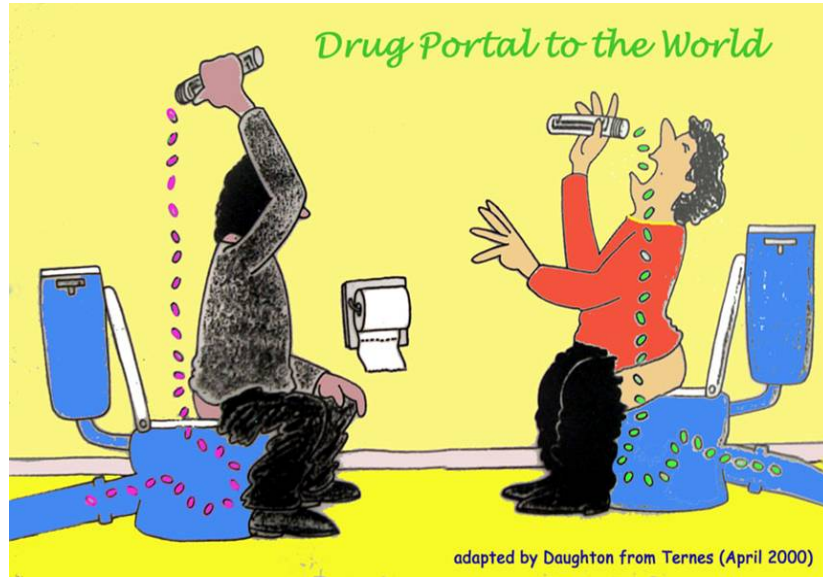


**Amy Pruden**

**W. Thomas Rice Professor**  
*Via Department of Civil &  
Environmental Engineering*



# How do antibiotics, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) end up in the environment?



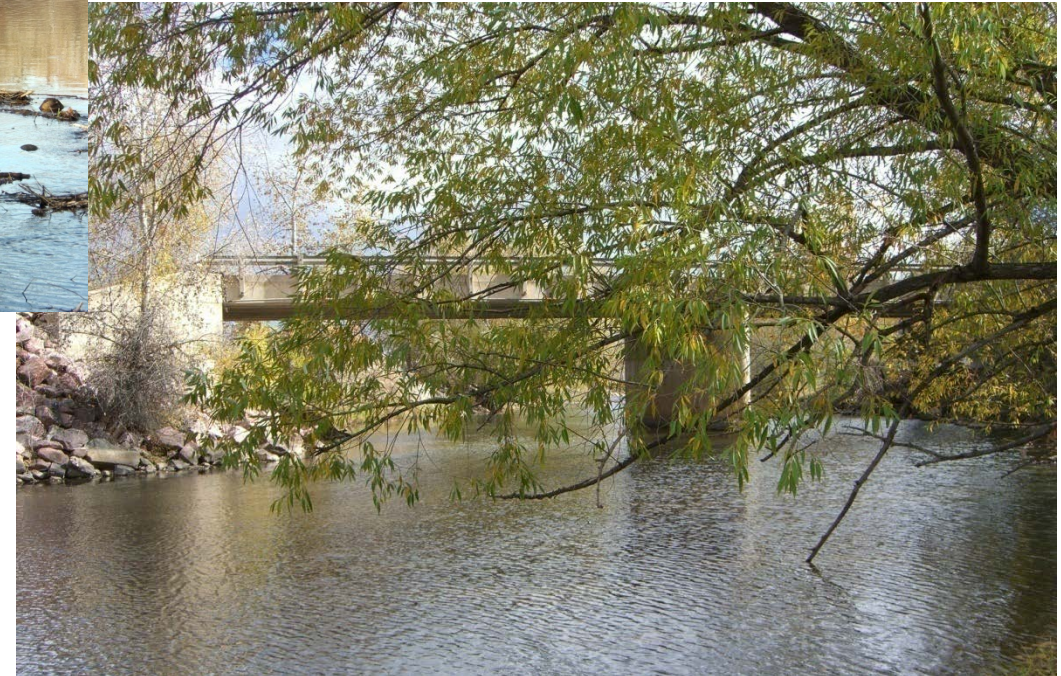
# Tracking Antibiotic Resistance Genes (ARGs) as Environmental “Pollutants”: *Poudre River Colorado*



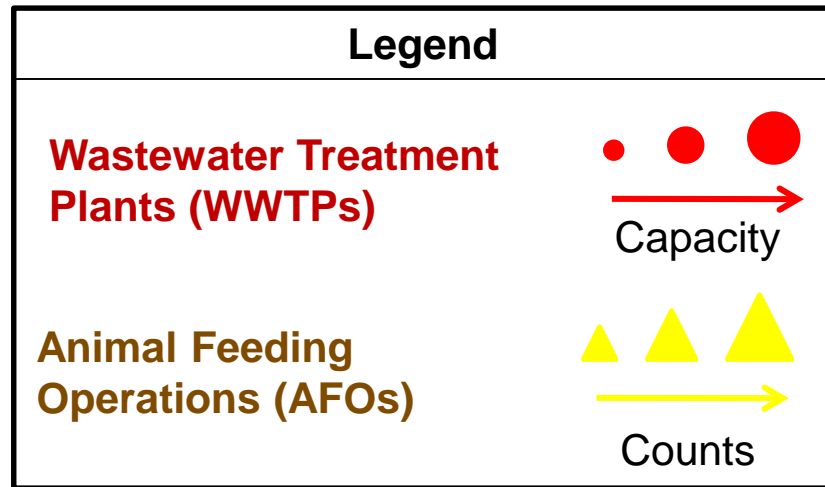
- Primary source is snowmelt from the Rocky Mountains



- Antibiotics measured in river consistent with defined zones of urban and agricultural influence

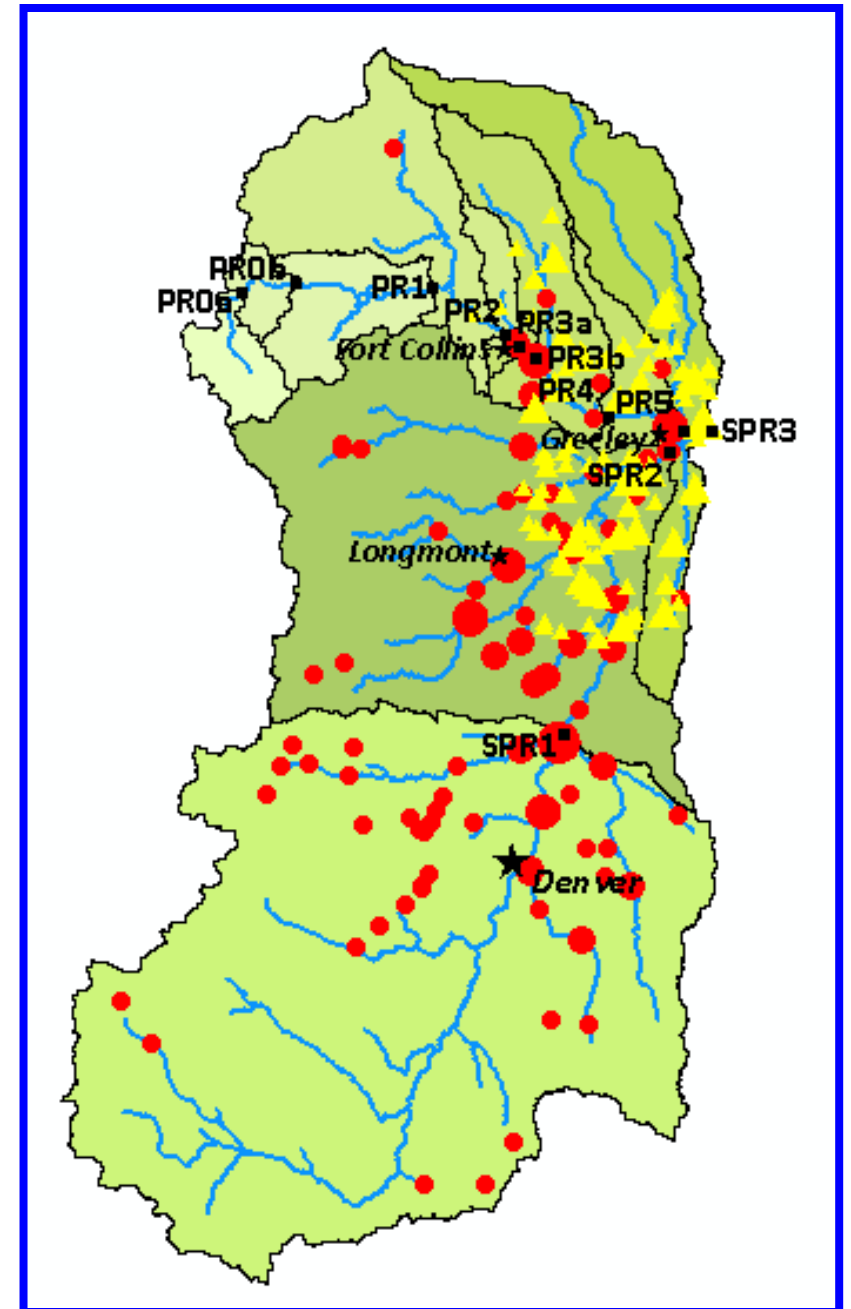


# Wastewater treatment plants and livestock operations in S. Platte and Poudre River watersheds

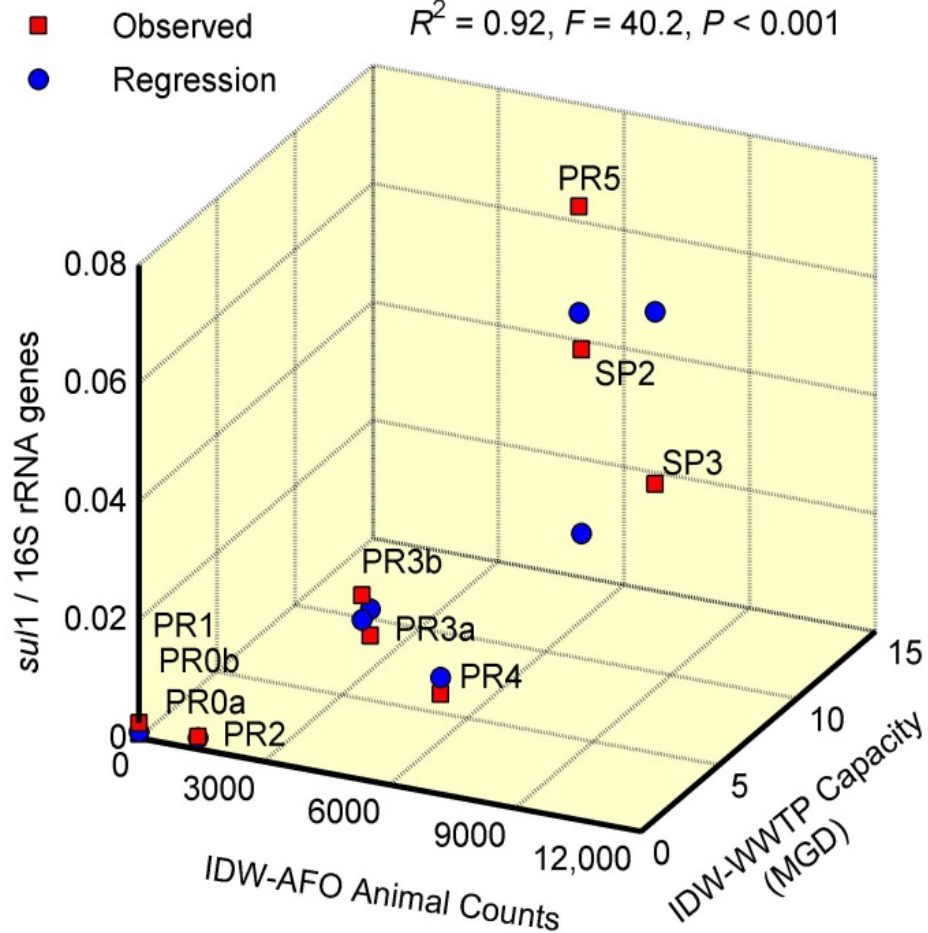


*\*IDW = Counts/capacities weighted by inverse distance from source to site*

Storteboom et al. *Environ. Sci. Technol.* 2010



# *sul1* ARGs

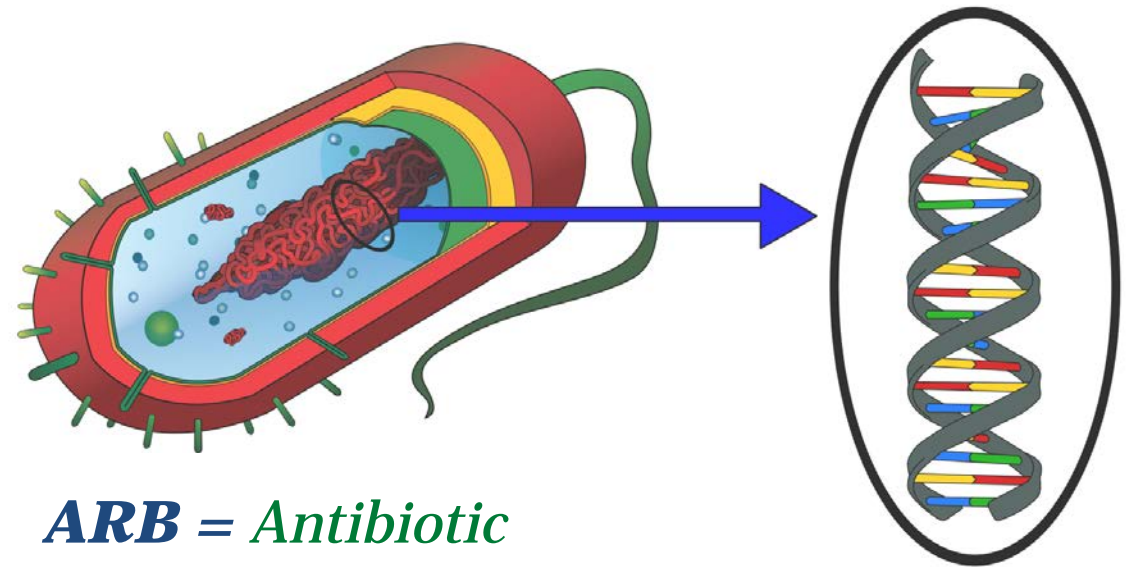


**( $R^2=0.92, p<0.001$ )!**  
**Multivariate linear regression model**  
Pruden et al. *Environ. Sci. Technol.* 2012

## *ARGs as*

### *“contaminants”*:

*sul1* ARGs (sulfonamide resistance) strongly correlated with upstream WWTPs and AFOs



**ARB = Antibiotic Resistant Bacteria**

**ARG = Antibiotic Resistance Gene**

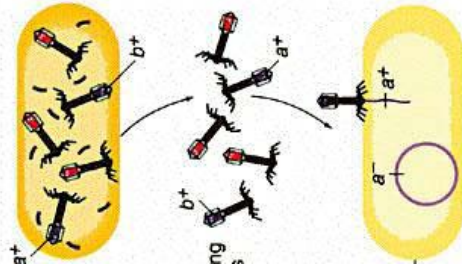
Mariana Ruiz Villarreal-  
Wikimedia Commons

# Targeting ARGs as “Contaminants”

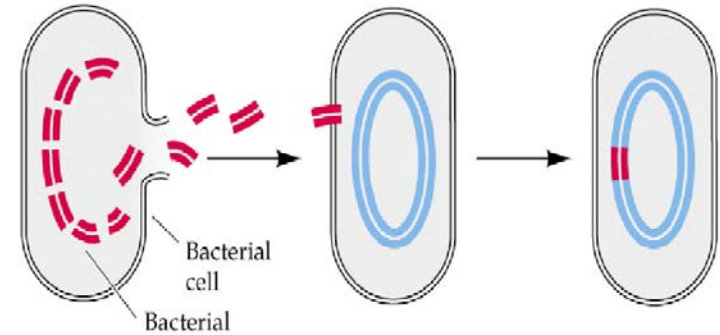
**Horizontal Gene Transfer:** Bacteria can share and spread ARGs



**Conjugation:**  
Bacterial “**mating**”



**Transduction: Virus**  
Mediated



**Transformation:** DNA from  
**dead** bacteria → **live** bacteria

- 1.) *Advantage in targeting ARGs directly- avoid culture bias, assess full microbial community*
- 2.) *BUT, must distinguish anthropogenic sources from BACKGROUND ARGs in experimental design*
- 3.) *“Contaminants” that can multiply*

# Antimicrobial resistance and the environment

The environment is key to antibiotic resistance. Bacteria in soil, rivers and seawater can develop resistance through contact with resistant bacteria, antibiotics, and disinfectant agents released by human activity. People and livestock can then be exposed to more resistant bacteria through food, water, and air.

**Human antibiotic use** jumped 36% in the 2000s



Up to **75% of antibiotics** used in aquaculture may be lost into the surrounding environment



**70% of antibiotics** are used by animals

Manure fertilizers cause antibiotic contamination in surface runoff, groundwater and drainage networks

**Antimicrobial use** for livestock will jump 67% by 2030

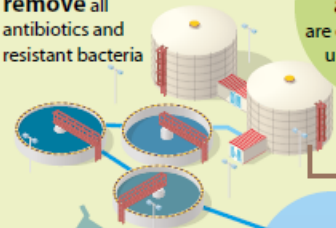
Antibiotics are increasingly used to boost animal growth in intensive farming, especially in developing countries

Antibiotics can be absorbed by plants and crops



**Major waste flows** including wastewater, manures and agricultural run-off contain antibiotic residues and antibiotic-resistant bacteria

Wastewater treatment plants **cannot remove** all antibiotics and resistant bacteria



Up to **80% of consumed antibiotics** are excreted through urine and faeces

**30% of antibiotics** are used by humans

Antibiotic resistant bacteria may be present in **raw source water** and **treated drinking water**



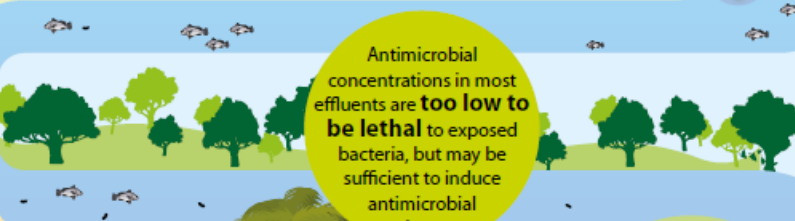
A vast array of **contaminants in municipal and industrial wastewater** increases pressure on bacteria to become resistant



**More than 50% of municipal solid waste** ends up in landfills and open dumps. This can include unused or expired drugs.



Antimicrobial concentrations in most effluents are **too low to be lethal** to exposed bacteria, but may be sufficient to induce antimicrobial resistance



**Multi-drug resistant bacteria** are prevalent in marine waters and sediments in close proximity to aquaculture, industrial and municipal discharges



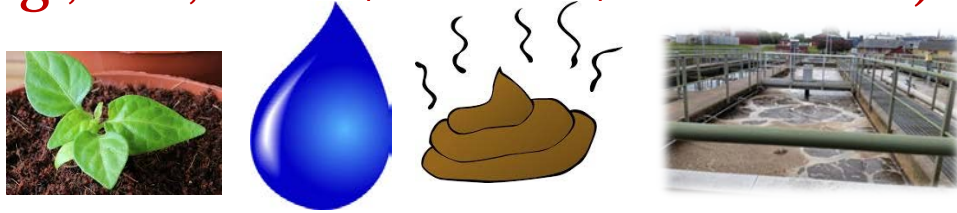
## FRONTIERS 2017

Emerging Issues of Environmental Concern



# From qPCR to Metagenomics

Environmental sample  
(e.g., soil, water, manure, wastewater)



Quantitative Polymerase  
Chain Reaction (qPCR)



[www.bio-rad.com](http://www.bio-rad.com)

## Target Select Genes:

*Indicators-*

- *Sul1*
- *intI1*

*Total Bacteria-*

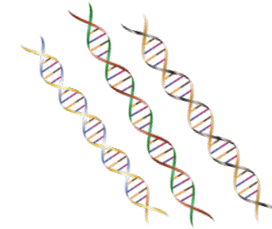
- 16S rRNA

*Clinically-Important-*

- *bla<sub>NDM-1</sub>*
- *qnrA*
- *vanA*
- *mcr1*



DNA Extraction



Shotgun Metagenomic  
Sequencing

## Target ALL Genes:

- *Depends on sequencing depth*
- *Higher detection limit*
- *Less quantitative*

MetaStorm

*Compare to Databases-*

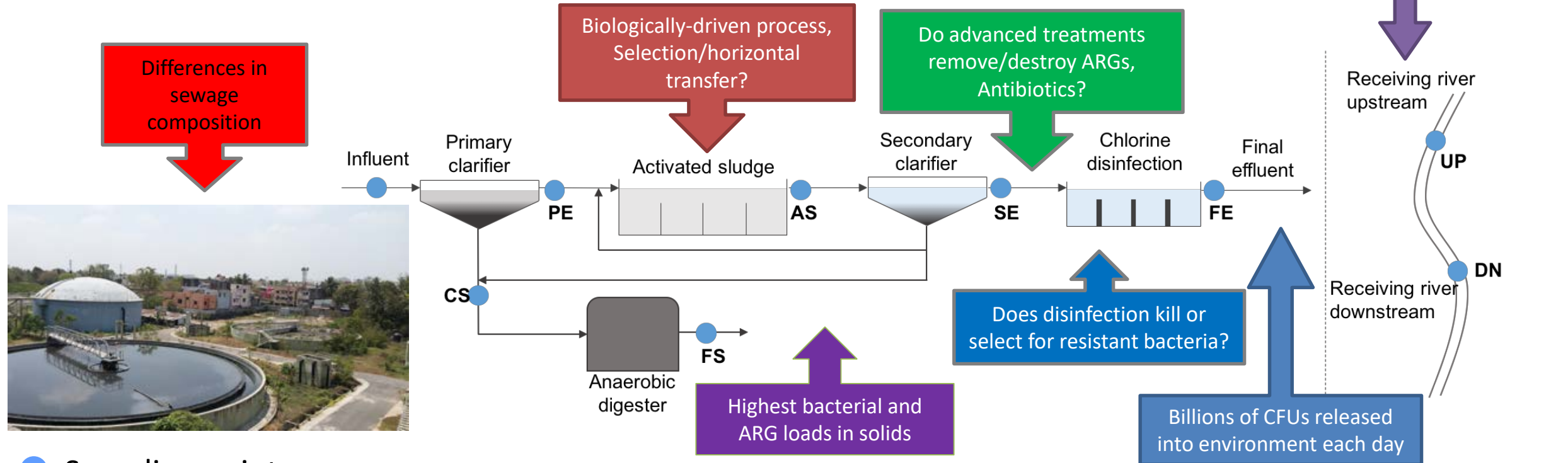
- CARD, Deep ARG
- ACLAME- Mobile genetic elements
- PATRIC- Pathogens
- BacMet- Metal resistance genes



[www.illumina.com](http://www.illumina.com)



# Effect of Wastewater Treatment: Standardizing Sampling for a Global Survey



● Sampling points

Effects of sample preservation and DNA extraction on enumeration of antibiotic resistance genes in wastewater

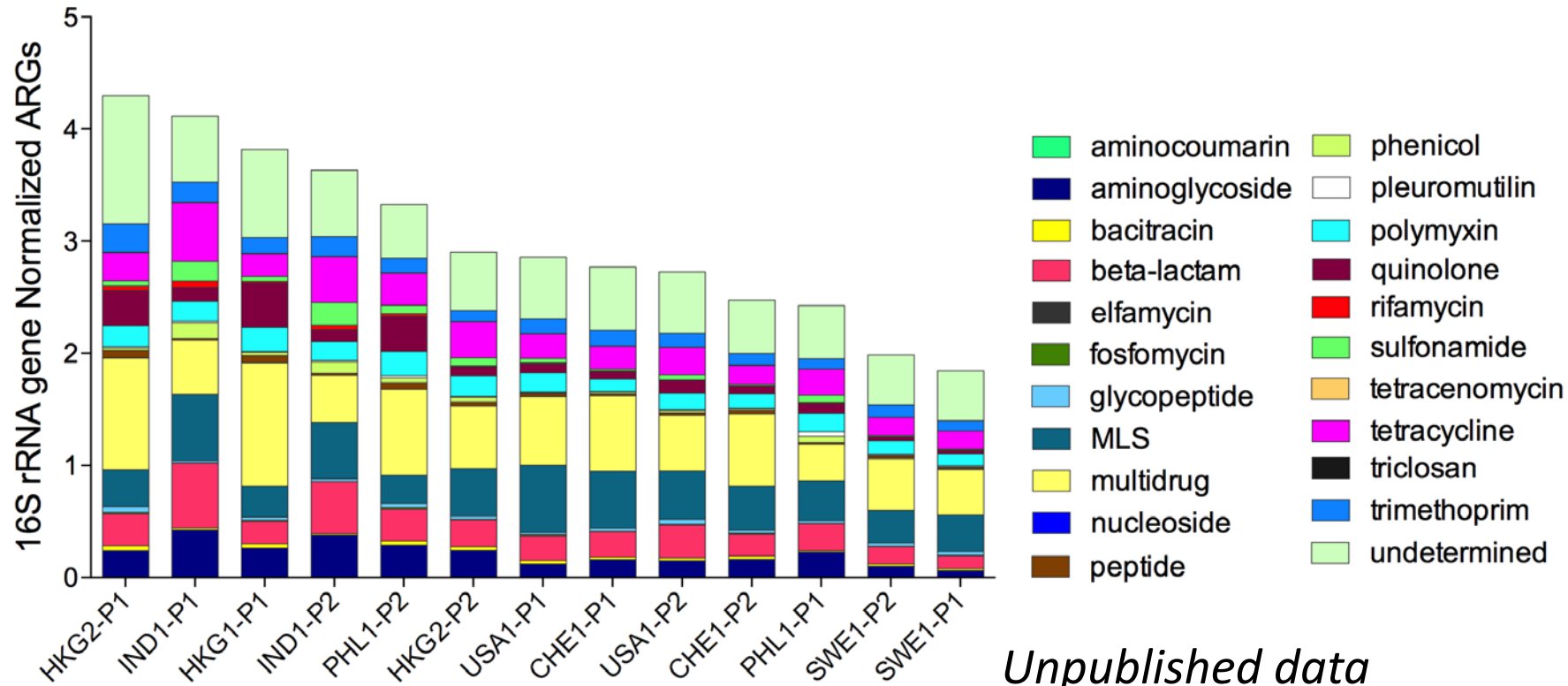
An-Dong Li<sup>1</sup>, Jacob W. Metch<sup>2</sup>, Yulin Wang<sup>1</sup>, Emily Garner<sup>2</sup>, An Ni Zhang<sup>1</sup>, Maria V. Riquelme<sup>2</sup>, Peter J. Vikesland<sup>2</sup>, Amy Pruden<sup>2,\*</sup> and Tong Zhang<sup>1,\*</sup>



**Partnership in International Research and Education (PIRE)**

# Global Metagenomic ARG Survey

## INFLUENT Sewage: Ranked “Total ARG” Abundance



-Highest in Hong Kong and India  
-Lowest in Sweden

CARD

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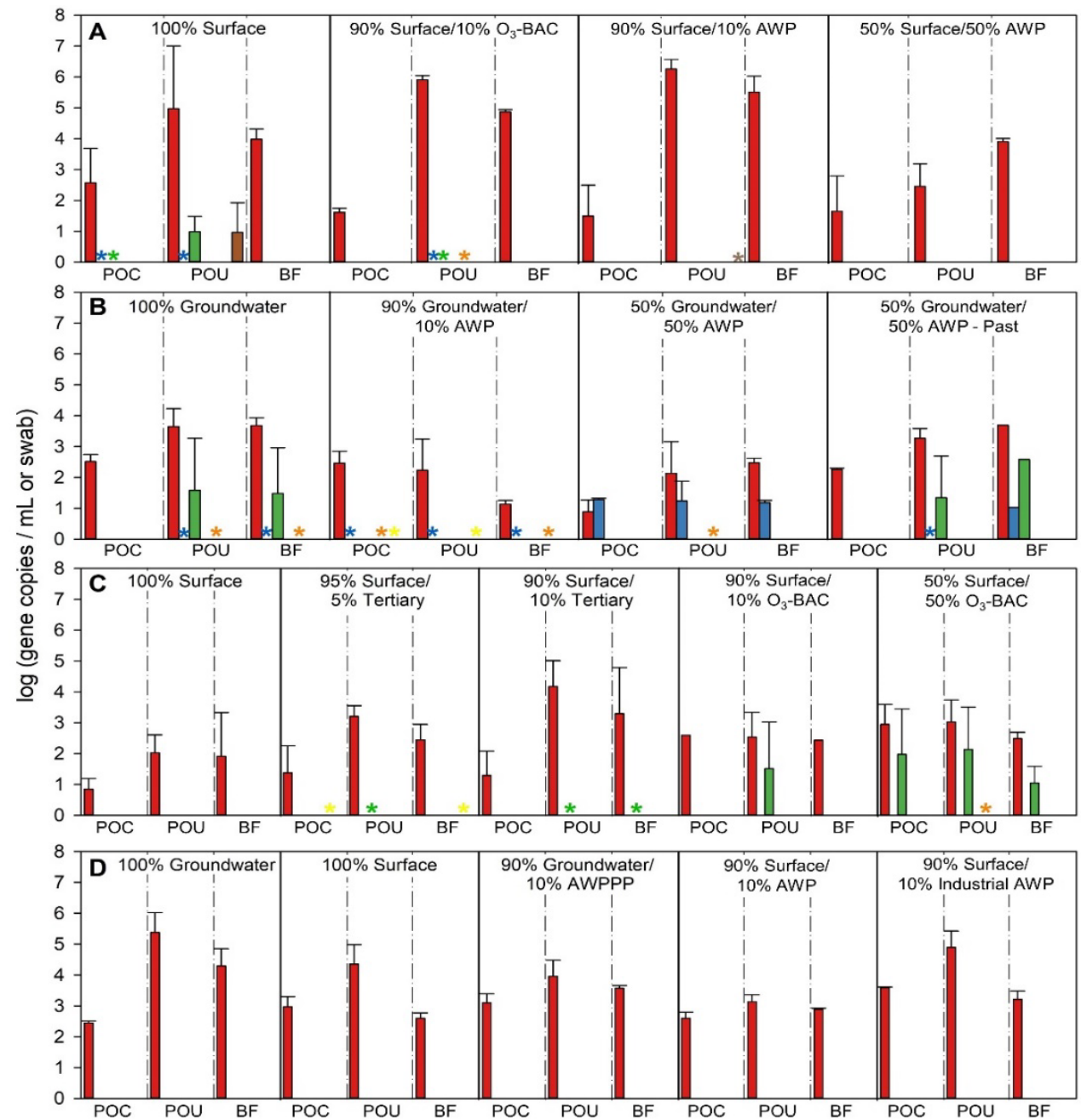
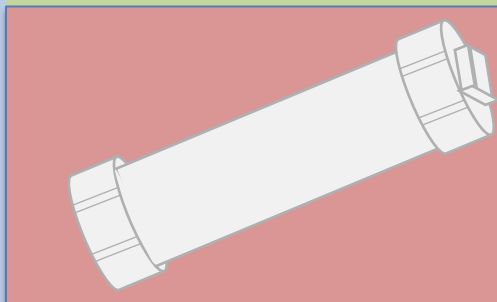
# Very low detection of pathogen and ARG markers (qPCR) after advanced water treatments and pipe incubation

**Total Bacteria-  
16S rRNA genes**

**Pathogens-  
*Legionella*  
*Mycobacteria*  
*P. aeruginosa***

**Antibiotic  
Resistance-  
*intI1*  
*qnrA*  
*vanA***

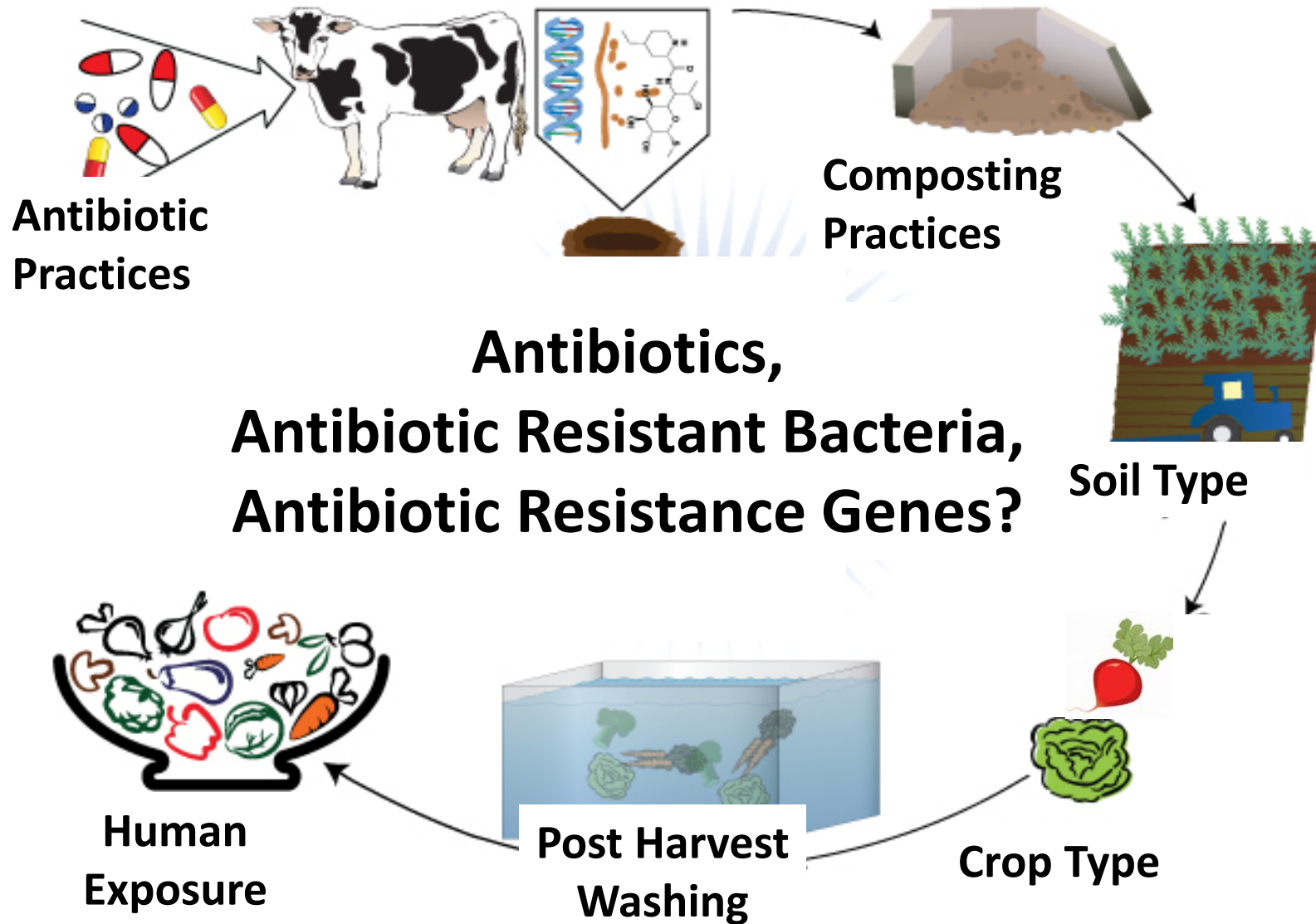
*Direct Potable Reuse Blends produced by a range of water treatments, incubated 8 weeks in PVC pipes*



Garner et al., *in review*

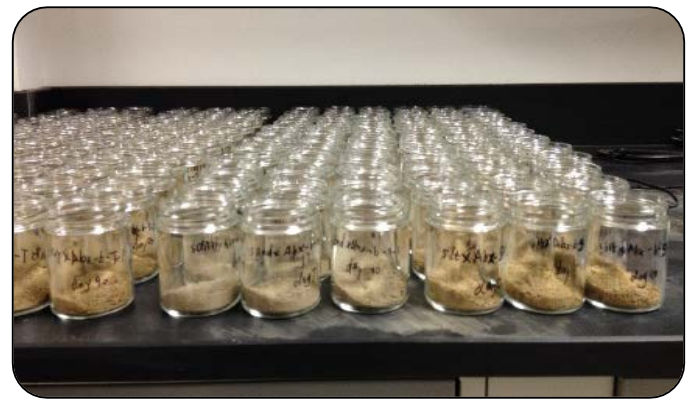
*\*detection too low to quantify*

# Critical Control Points for Mitigating AR from “Farm to Fork”

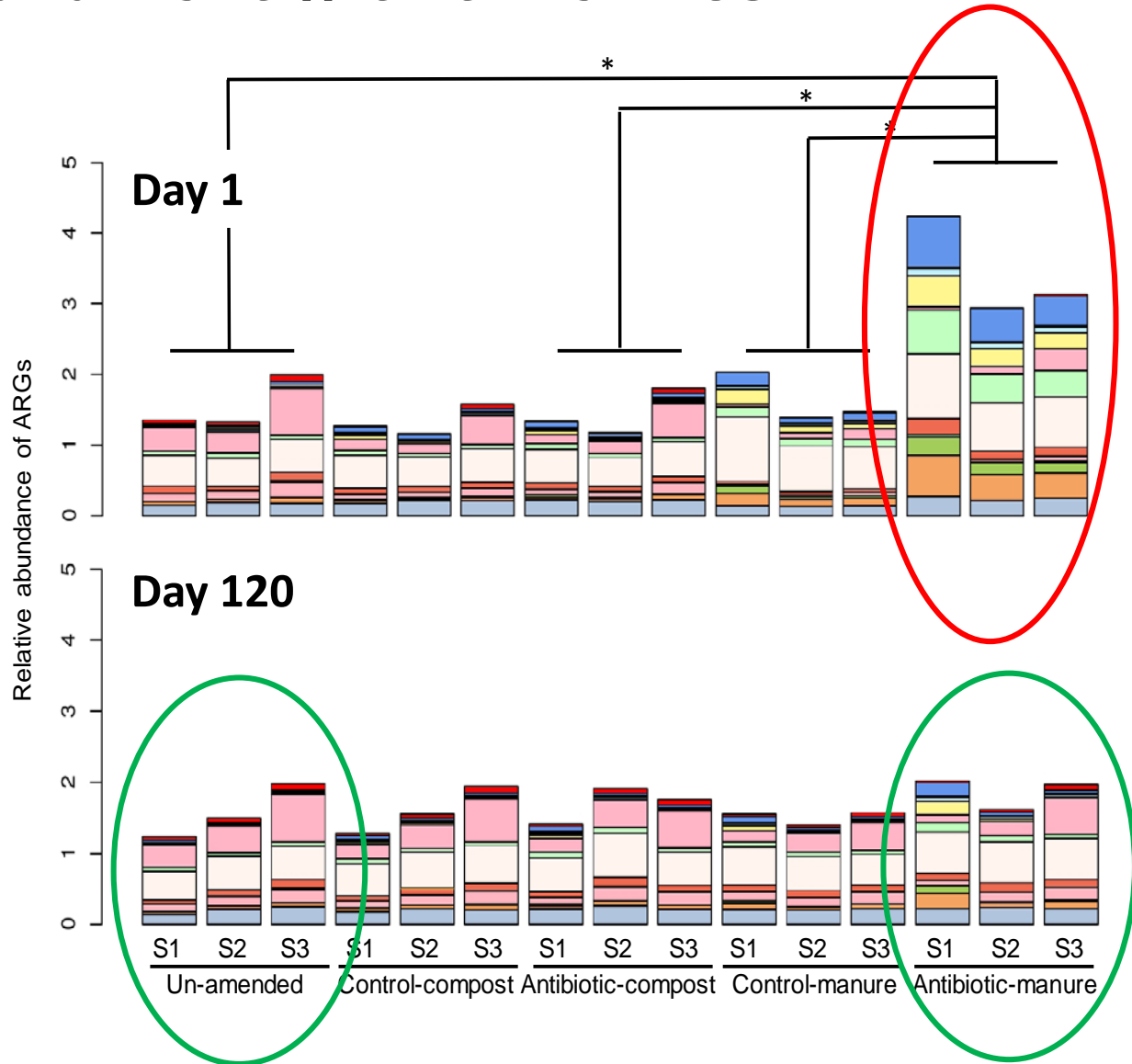


#2014-05280

# Total ARGs: Effect of compost vs dairy manure amendment with time in soil\*



Soil Microcosms



- **Total ARGs highest in dairy manure with Abx condition 1 day after mixing with soil**
- **After 120 days, total ARGs equivalent to the no amendment background condition, but different signature**

**120 Days:** USDA organic guidelines- manure app-> harvest

- S1 Loamy Sand
- S2 Silt Loam
- S3 Silty Clay Loam

\*  $n=3$ , ANOSIM  $p<0.05$

# MetaCompare: A Computational Pipeline for Ranking “Resistome Risk” in Various Environmental Compartments

An assembled Contig

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

Genetic Identity against ARGs

**CARD**

no → No resistome risk

yes

Mobile Genetic Element?

**ACLAME**

no → Less mobility but holds low risk

yes

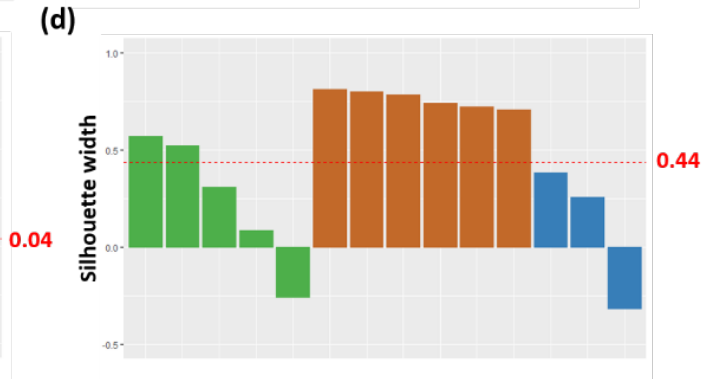
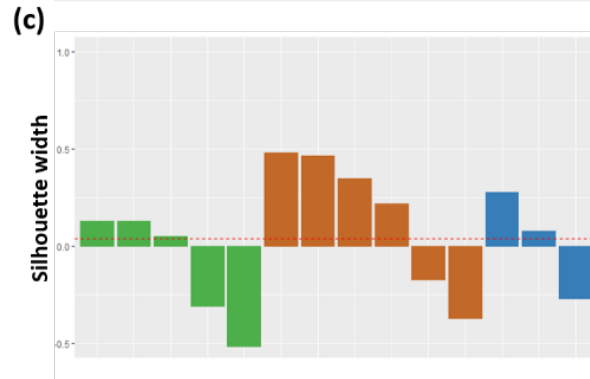
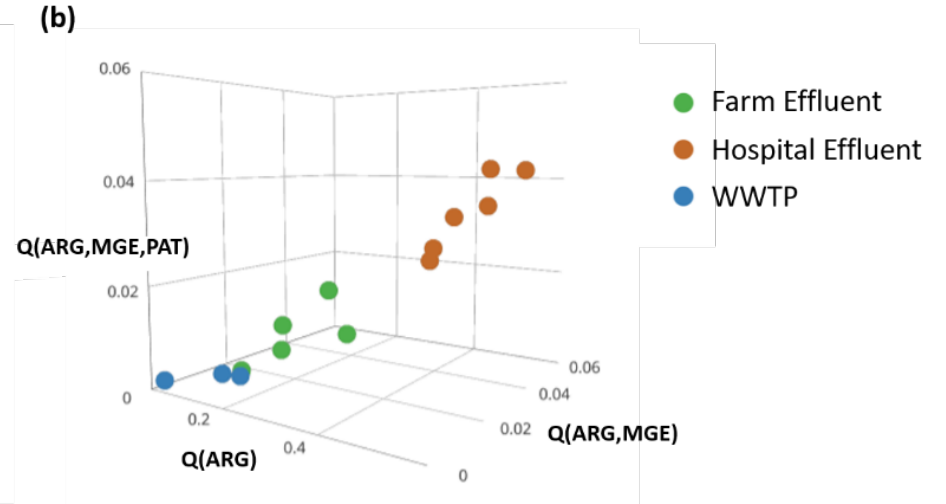
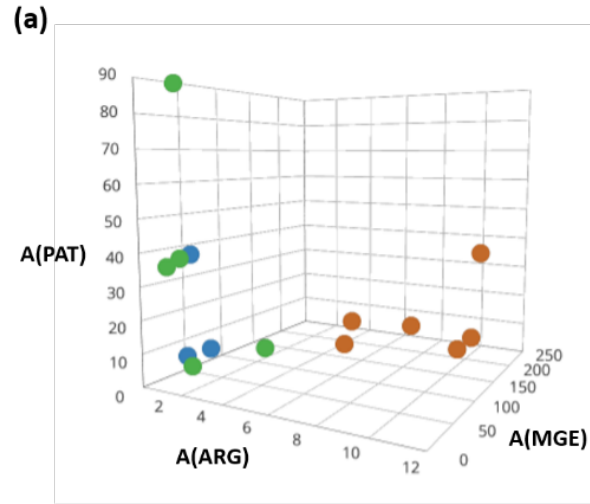
Human Pathogen?

**PATRIC**

no → Less infectious but high mobility

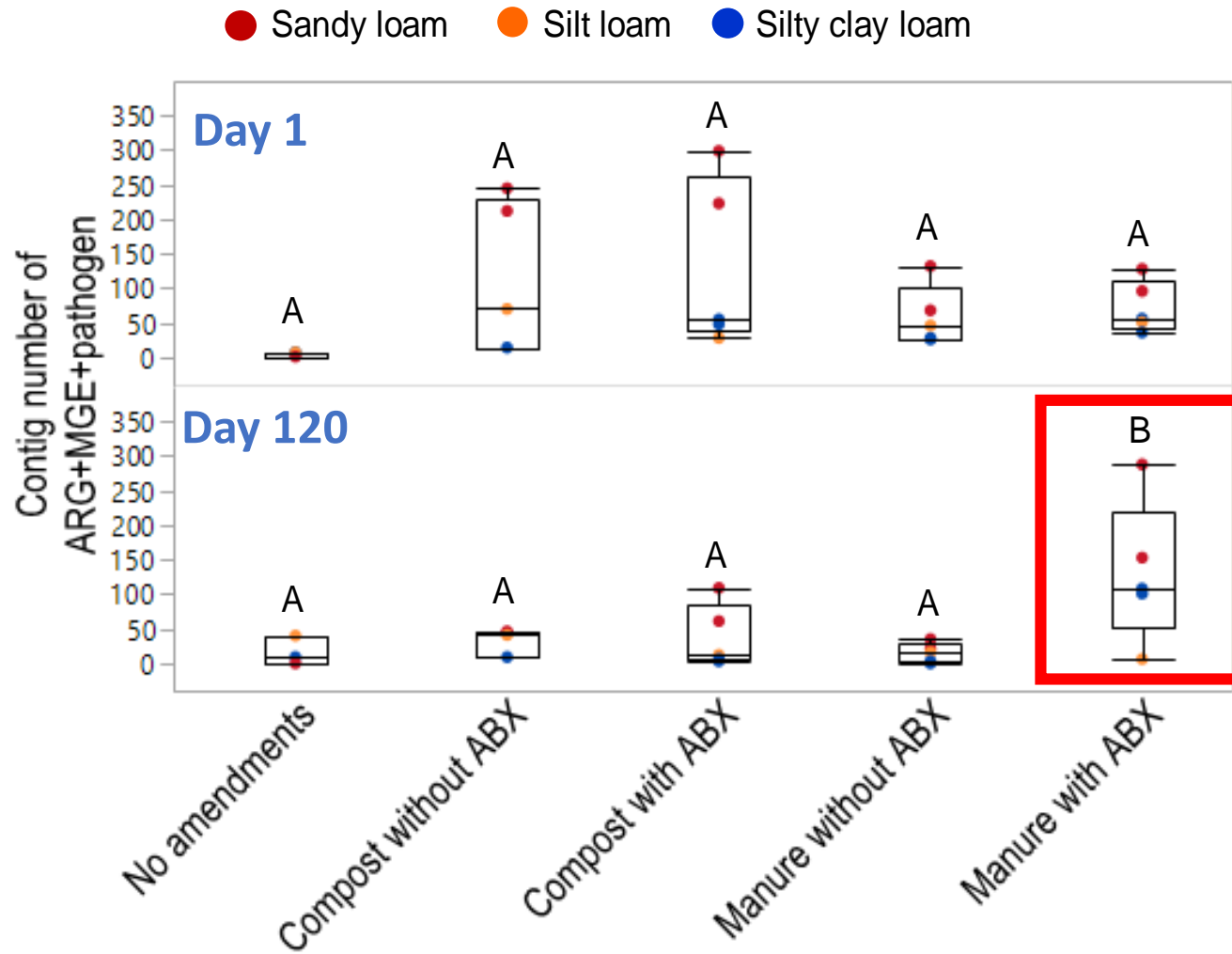
yes

Highly Infectious with high Mobility



Adapted from: Martínez, Coque, and Baquero. "What is a resistance gene? Ranking risk in resistomes." *Nature Reviews Microbiology* 13.2 (2015): 116-123.

# # Contigs with ARG + MGE + pathogen: Resistome Risk of three soils amended with dairy manure or compost

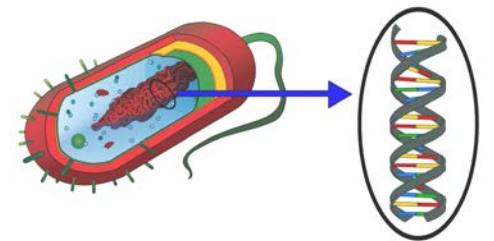


Soil Microcosms

Ranked “resistome risk” remains elevated in soils amended with dairy manure with antibiotics at 120 days

# Take Home Messages

- We need to commence formal monitoring of antimicrobial resistance in the environment!
  - Establish “baseline/background”
  - Appropriate sampling, statistical design
  - Inform risk frameworks, assessment, and management
- We have targets here and now that can be measured- with trends that make sense, e.g.:
  - Total ARGs
  - Clinically-important ARGs
  - ARGs on mobile elements/pathogens (resistome risk)
  - Complement culture-based monitoring e.g., WHO AGISAR ESBL *E. coli*
- Need to work together with policy makers towards consensus on *what, where, when* to monitor







Andy Salvesson, Carollo



Jeannie McLain, U AZ



Charles Bott, HRSD



Katharine Knowlton



Monica Ponder



Leigh Anne Krometis



Cully Hession



Marc Edwards, VTech



Peter Vikesland, VTech

### Water Reuse

### Food and Ag

### Water Chemistry, Pipes, Wastewater, Sensors



Kang Xia, VTech



Diana Aga, U at Buffalo



Liqing Zhang, VTech



Lenny Heath, VTech



Dave Engelthaler, TGen

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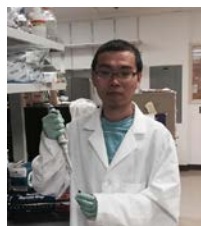


### Analytical Chemistry

### Bioinformatics



Maria Virginia Riquelme



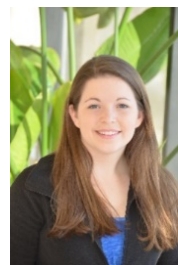
Chaoqi Chen



Dongjuan Dai



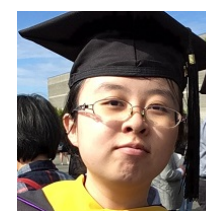
Min Oh



Emily Garner



Gustavo Arango Argoty



Xiao Liang

### Students/Post-docs



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.....STUDENTS AND COLLABORATORS