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

Assessment and Analysis

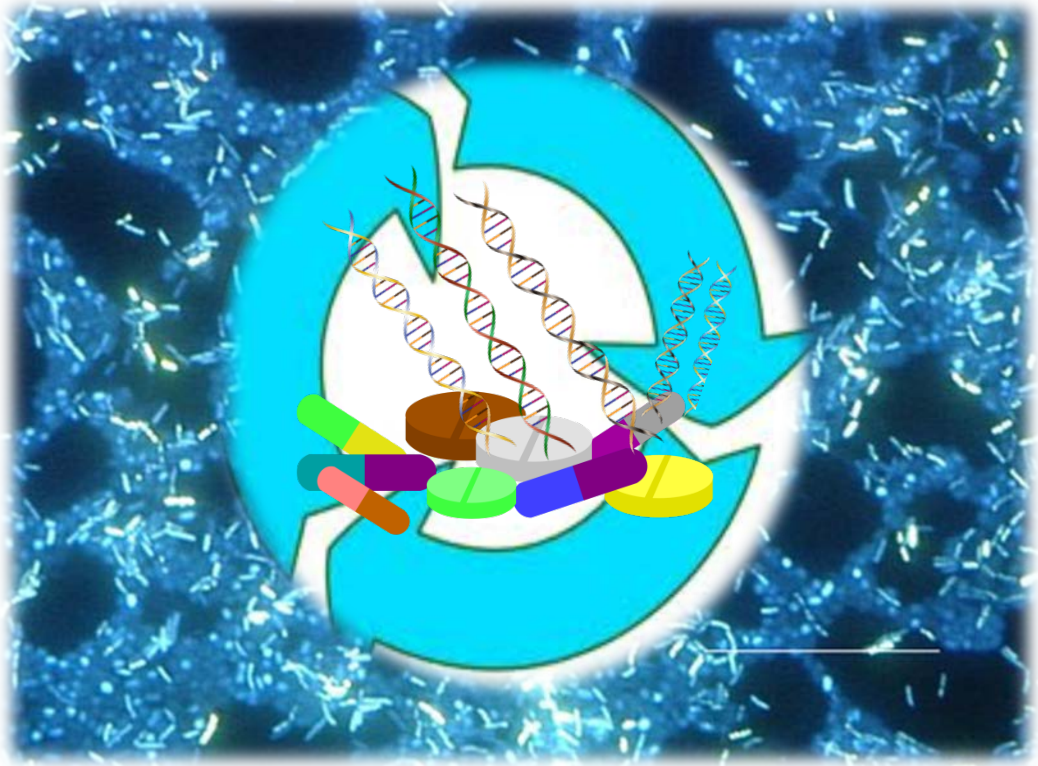


Image: Rodney M. Donlan, CDC

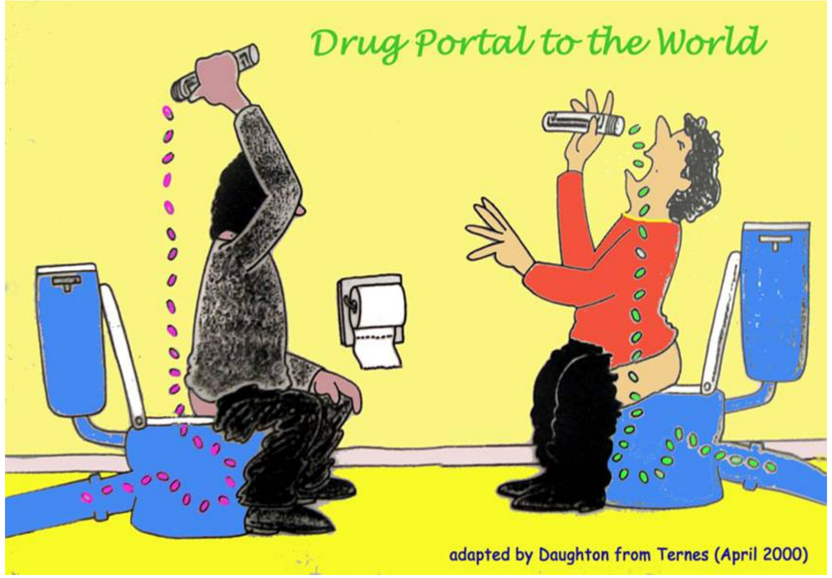
Amy Pruden

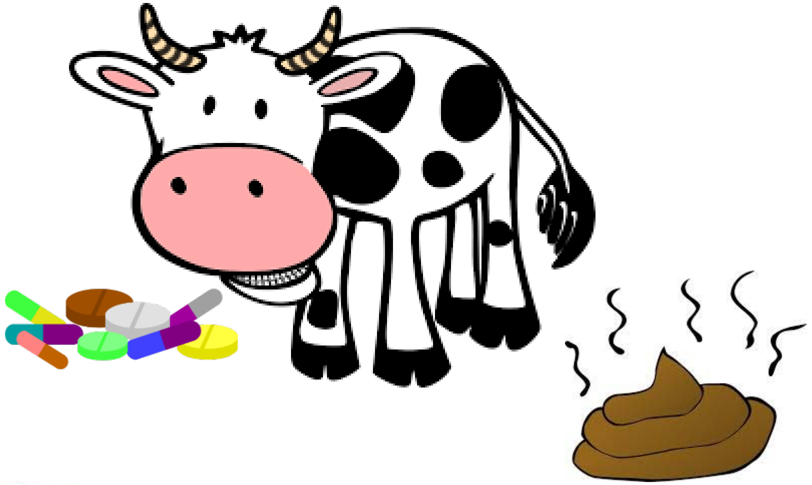
W. Thomas Rice Professor

Via Department of Civil & Environmental Engineering

VIRGINIA TECH

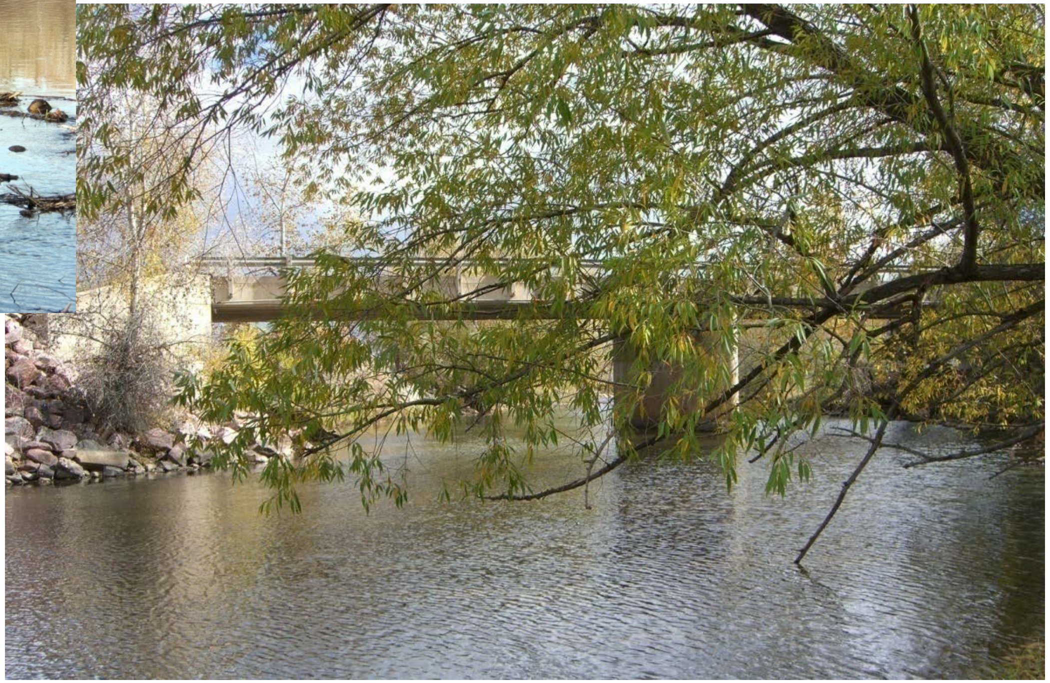
How do antibiotics, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) end up in the environment?





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

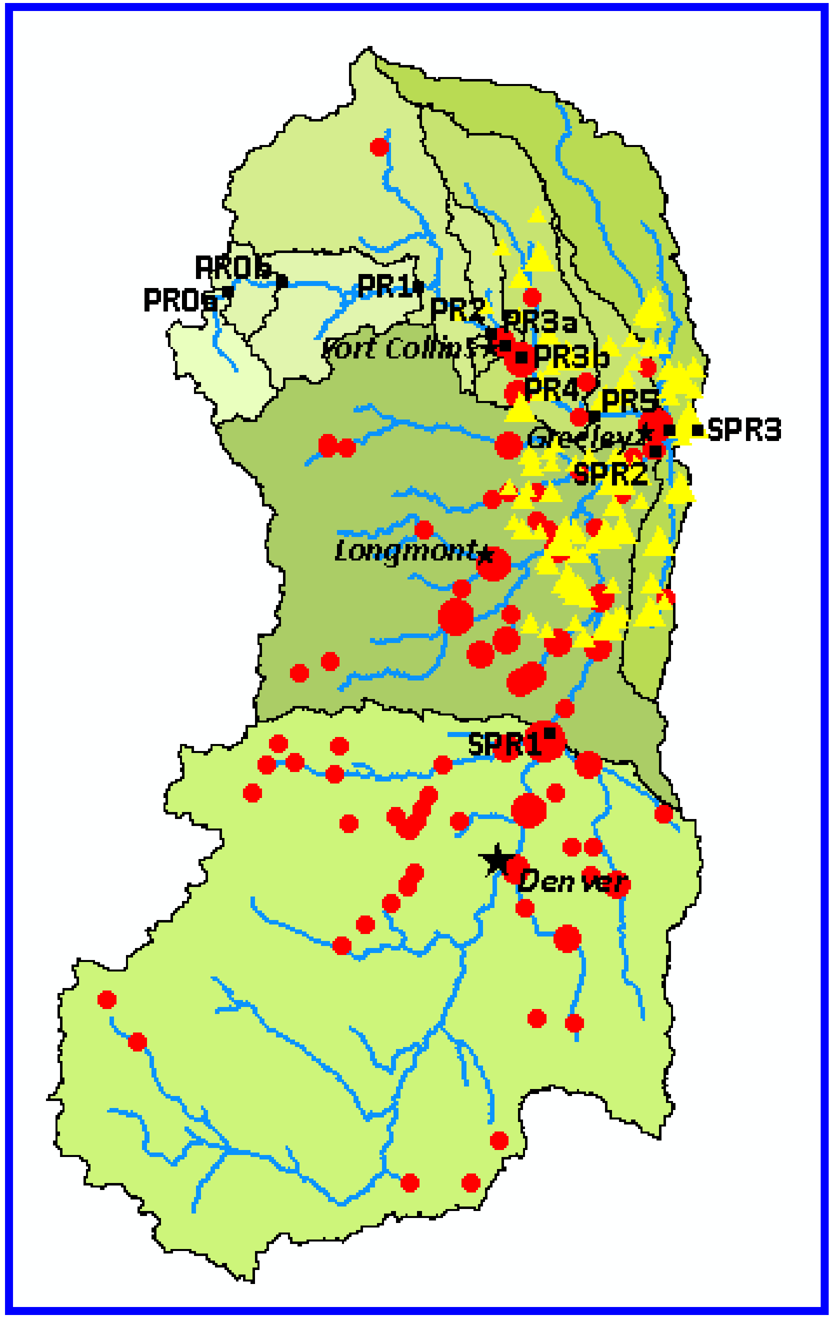
Wastewater treatment plants and livestock operations in S. Platte and Poudre River watersheds

Legend

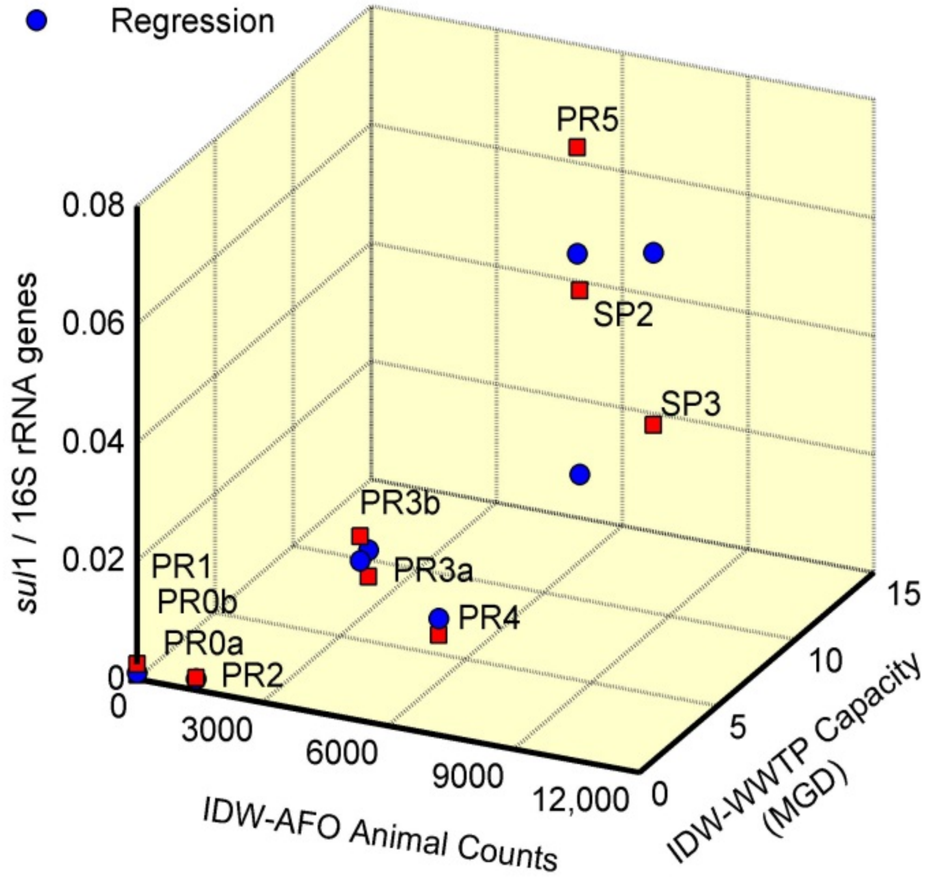
Wastewater Treatment Plants (WWTPs)	Capacity
Animal Feeding Operations (AFOs)	Counts

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

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



sul1 ARGs



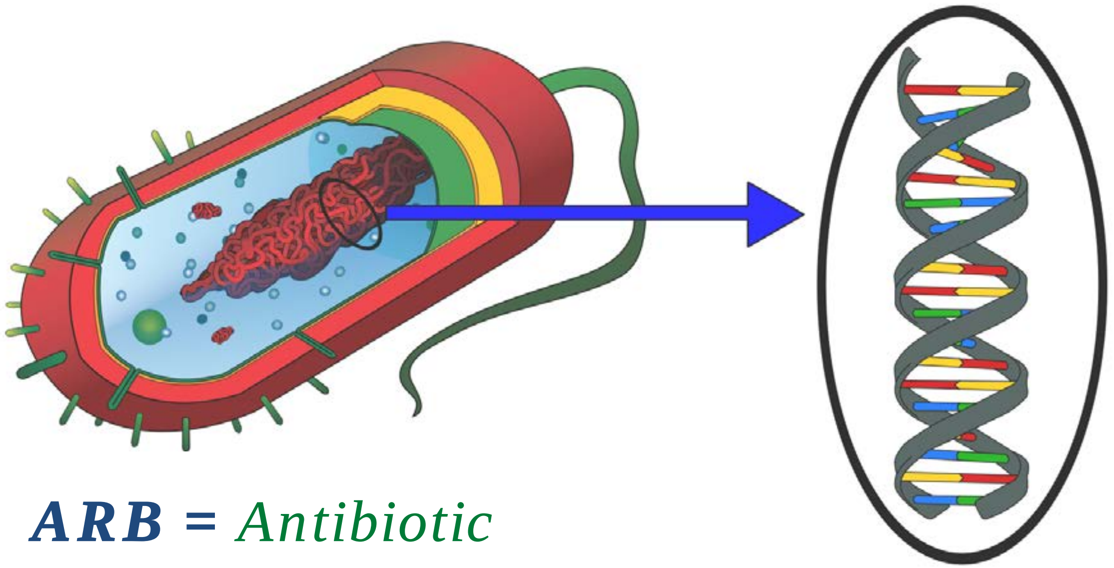
(R^2=0.92, p<0.001)!

Multivariate linear regression model

Pruden et al. *Environ. Sci. Technol.* 2012

ARGs as "contaminants":

*sul*1 ARGs (sulfonamide resistance) strongly correlated with upstream WWTPs and AFOs



***ARB** = Antibiotic Resistant Bacteria*

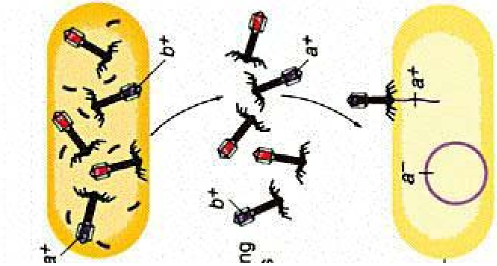
Mariana Ruiz Villarreal-Wikimedia Commons

***ARG**= Antibiotic Resistance Gene*

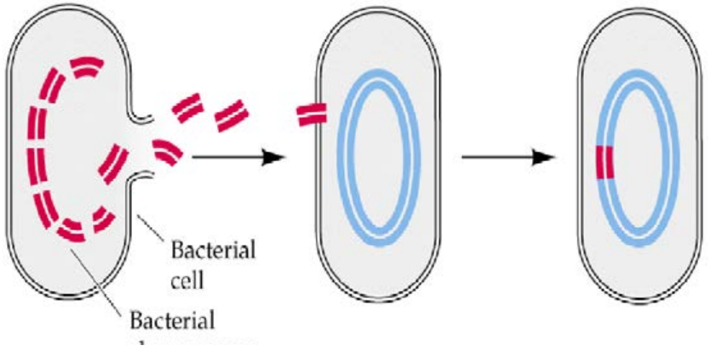
Targeting ARGs as "Contaminants"

Horizontal Gene Transfer: Bacteria can share and spread ARGs

Conjugation:
Bacterial "**mating**"



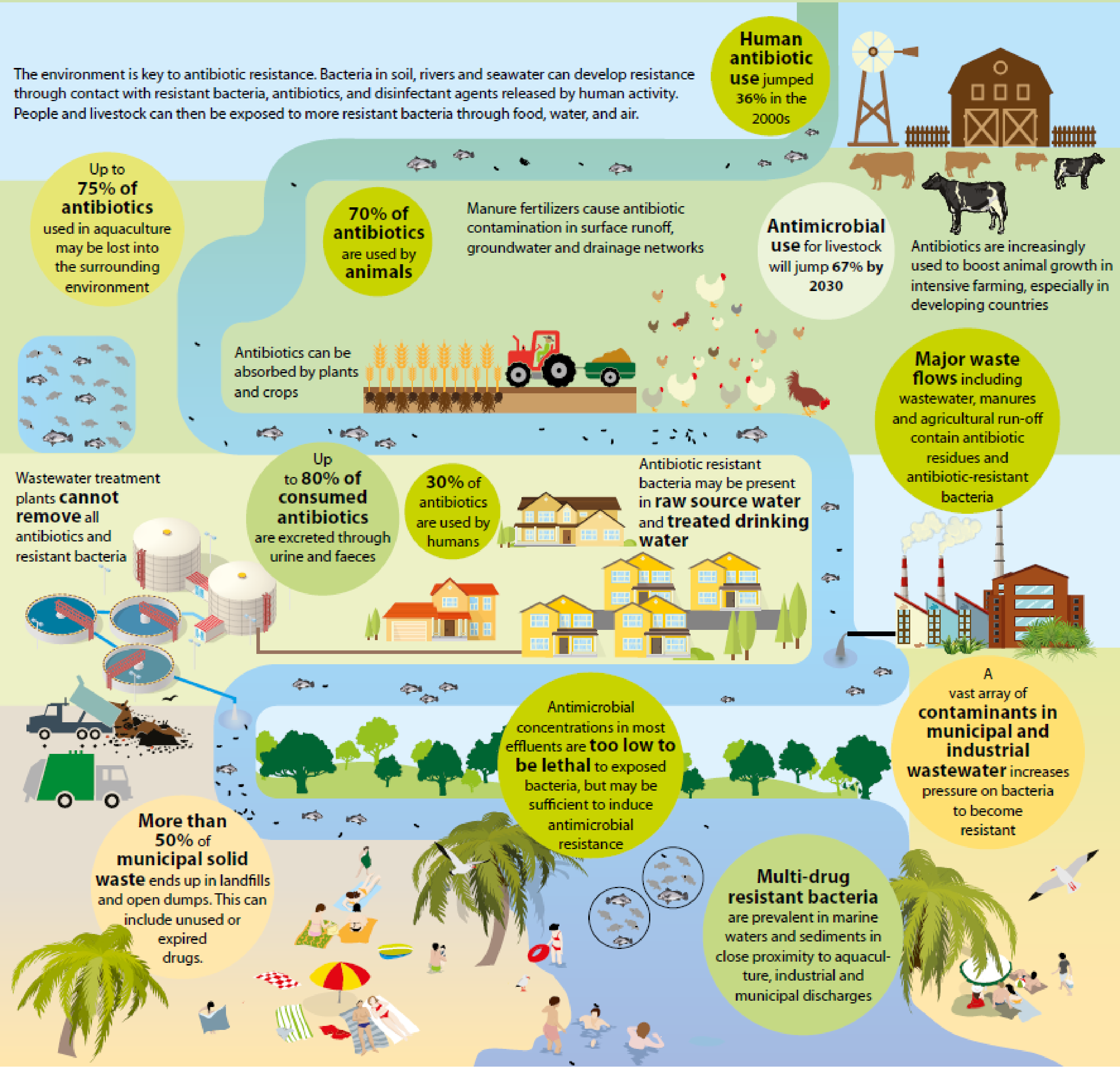
Transduction: Virus
Mediated



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



UN environment

FRONTIERS 2017

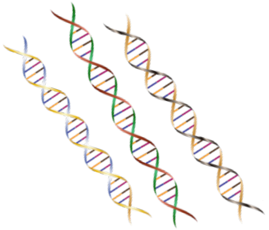
Emerging Issues of Environmental Concern

From qPCR to Metagenomics

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




DNA Extraction



Quantitative Polymerase Chain Reaction (qPCR)

www.bio-rad.com

Target SELECT Genes:
-Quantitative
-Sensitive

Target Select Genes:

Indicators-
- *Sul*1
- *int*I1

Total Bacteria-
- 16S rRNA

Clincially-Important-
- bla_{NDM-1}
- *qnr*A
- *van*A
- *mcr*1

Shotgun Metagenomic Sequencing

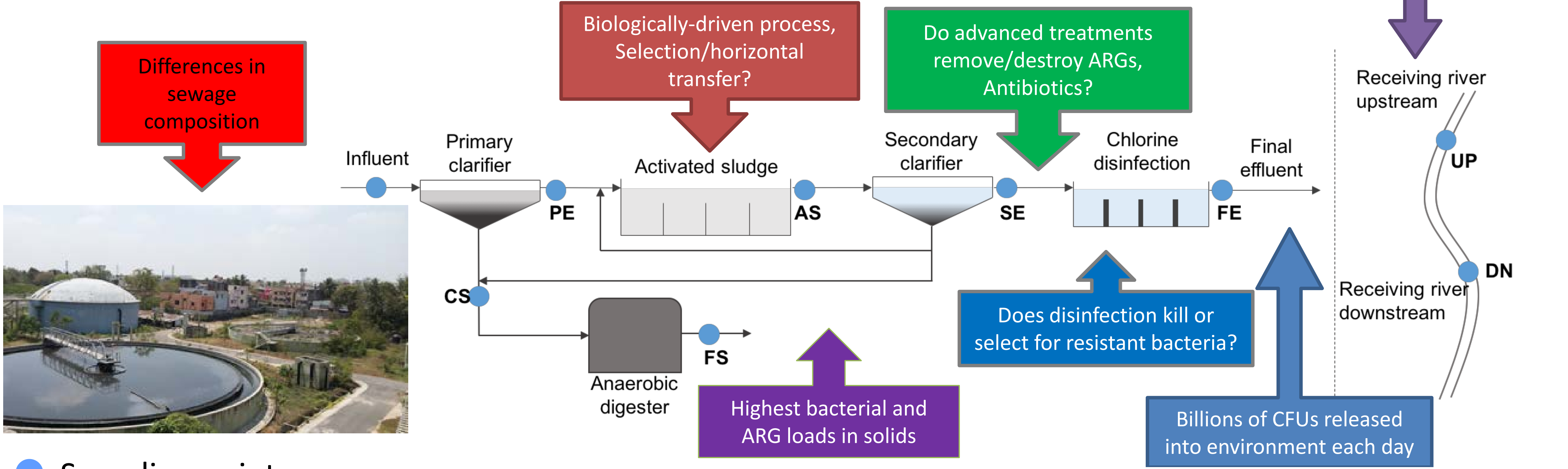
Target ALL Genes:
-Depends on sequencing depth
-Higher detection limit
-Less quantitative

www.illumina.com

MetaStorm

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

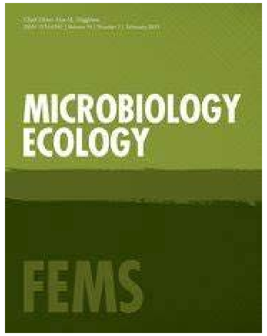
Effect of Wastewater Treatment: Standardizing Sampling for a Global Survey



Sampling points

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

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



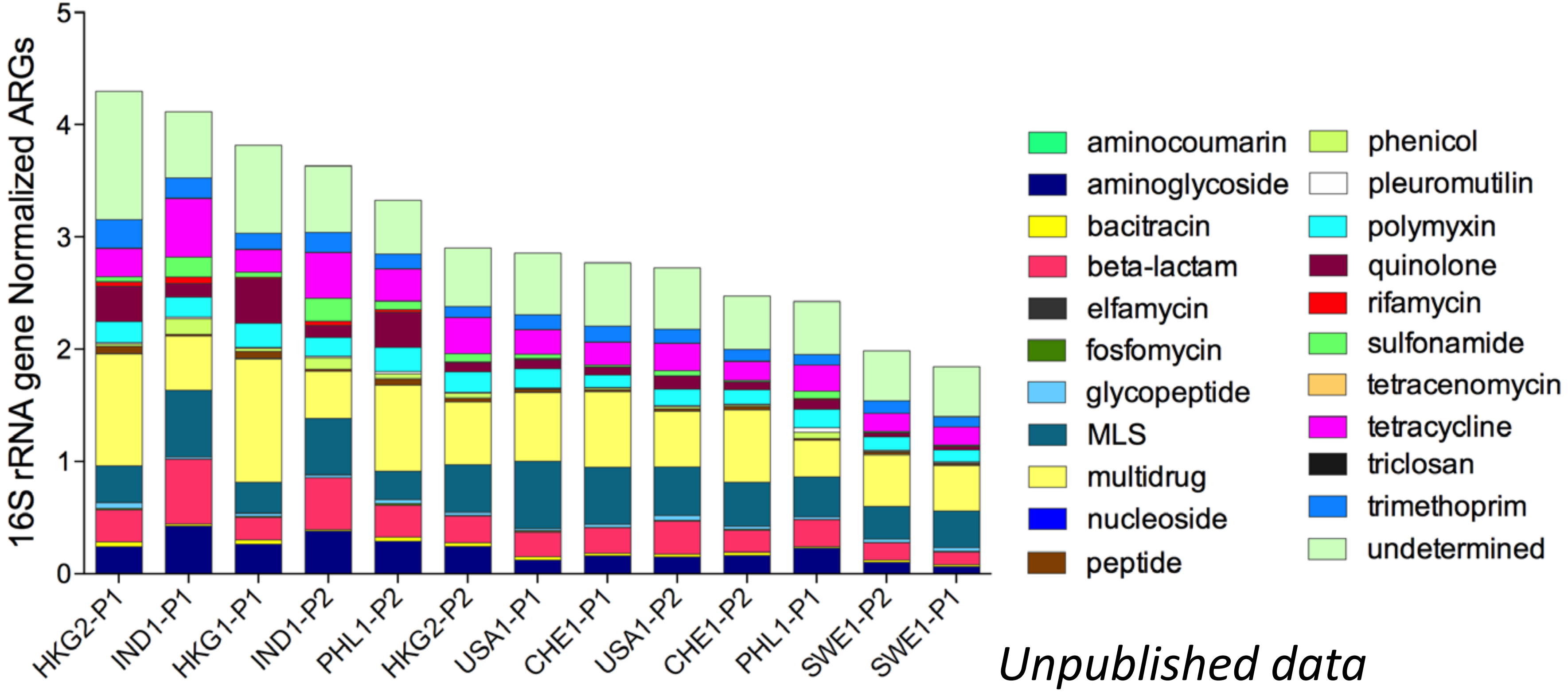
Halting Environmental Antimicrobial Resistance Dissemination

#1545756

Partnership in International Research and Education (PIRE)

Global Metagenomic ARG Survey

INFLUENT Sewage: Ranked "Total ARG" Abundance



Locations:

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

Unpublished data

-Highest in Hong Kong and India
-Lowest in Sweden

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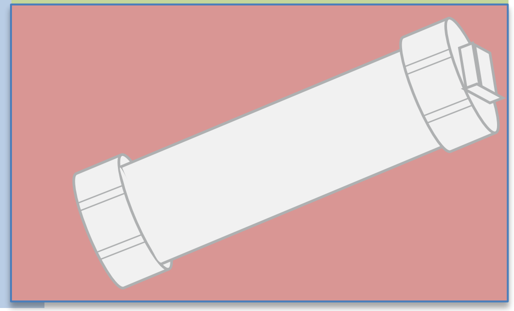
Very low detection of pathogen and ARG markers (qPCR) after advanced water treatments and pipe incubation

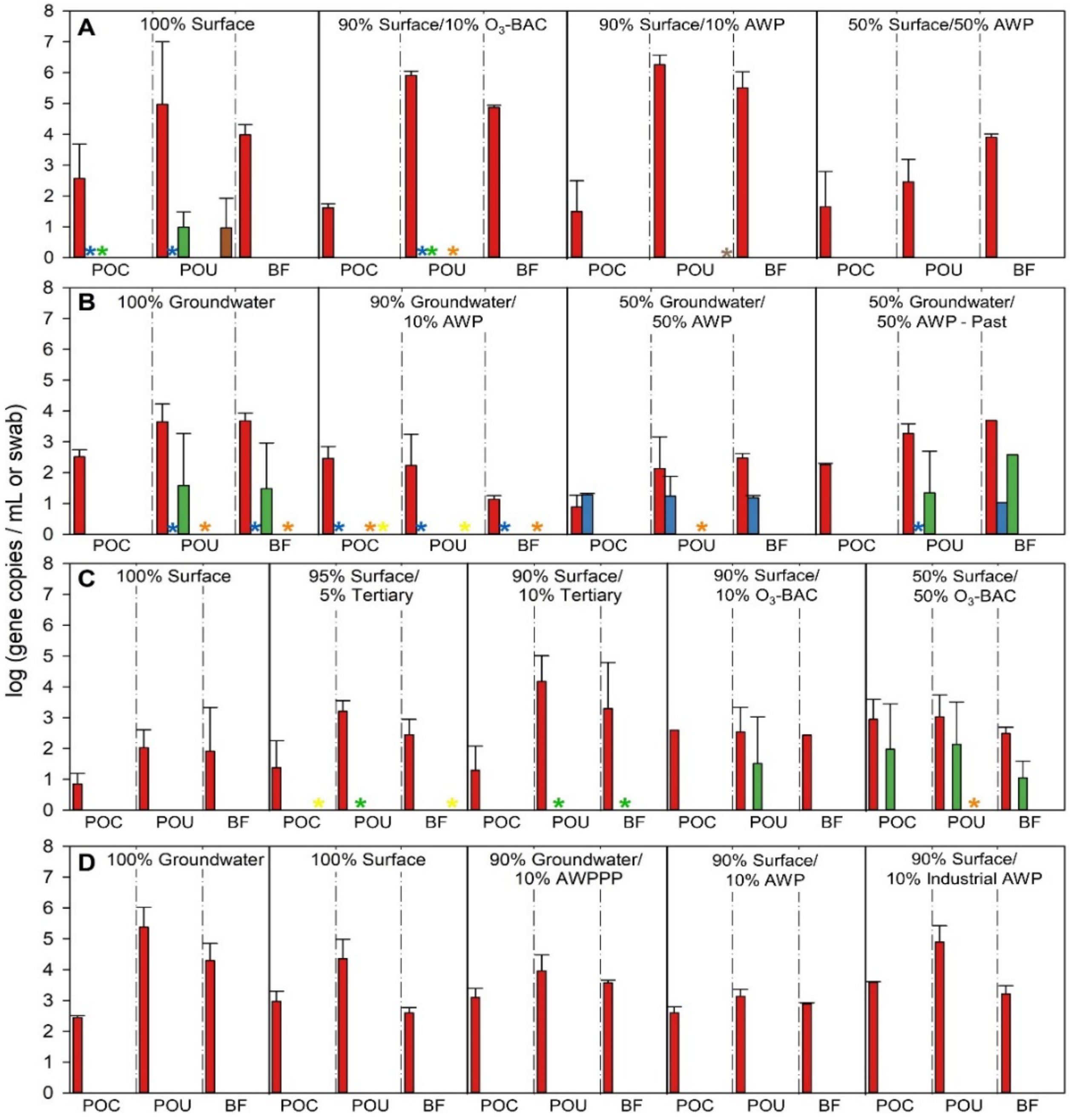
Total Bacteria-
16S rRNA genes

Pathogens-
Legionella
Mycobacteria
P. aeruginosa

Antibiotic Resistance-
*int*I1
*qnr*A
*van*A

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

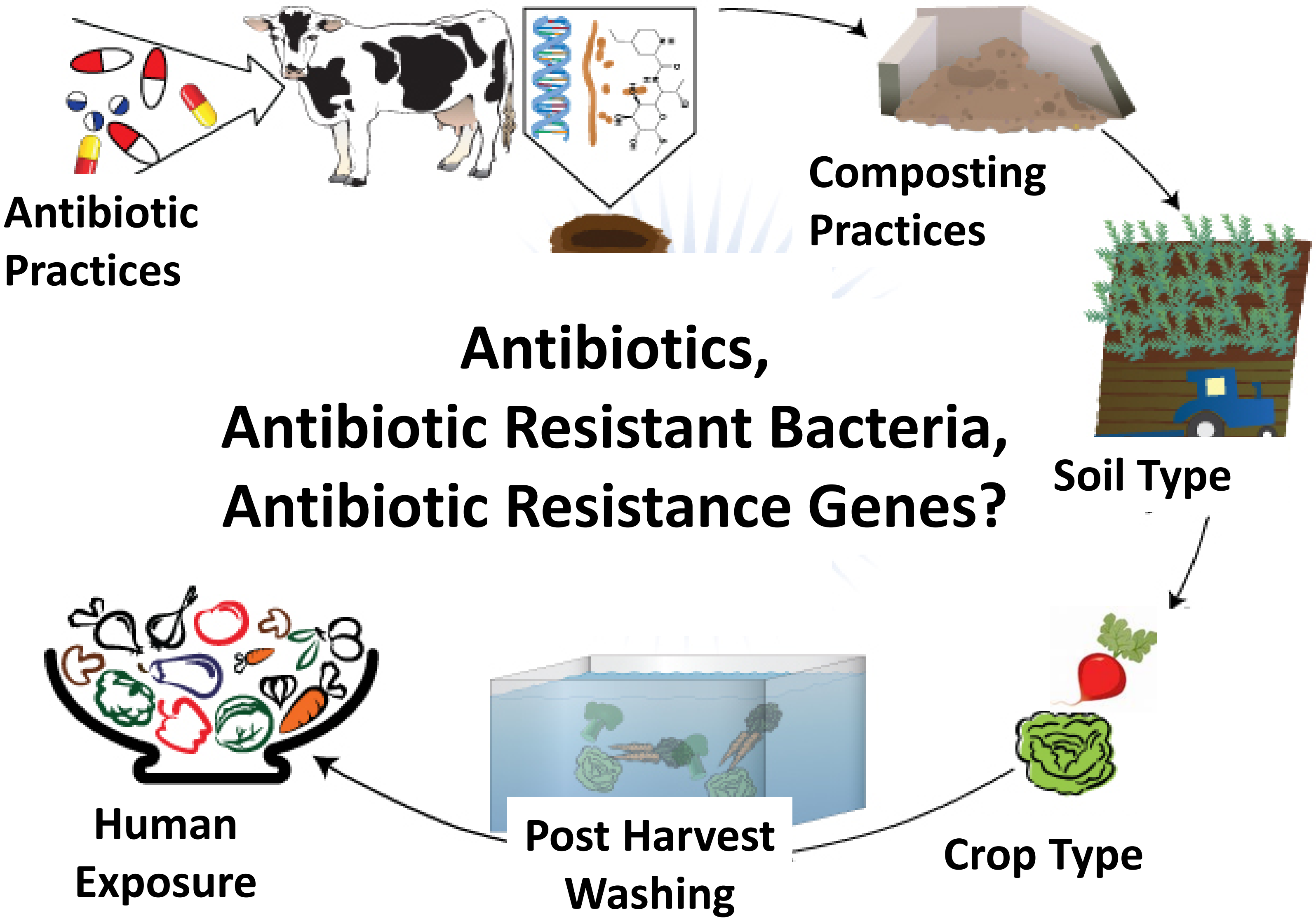




Garner et al., *in review*

**detection too low to quantify*

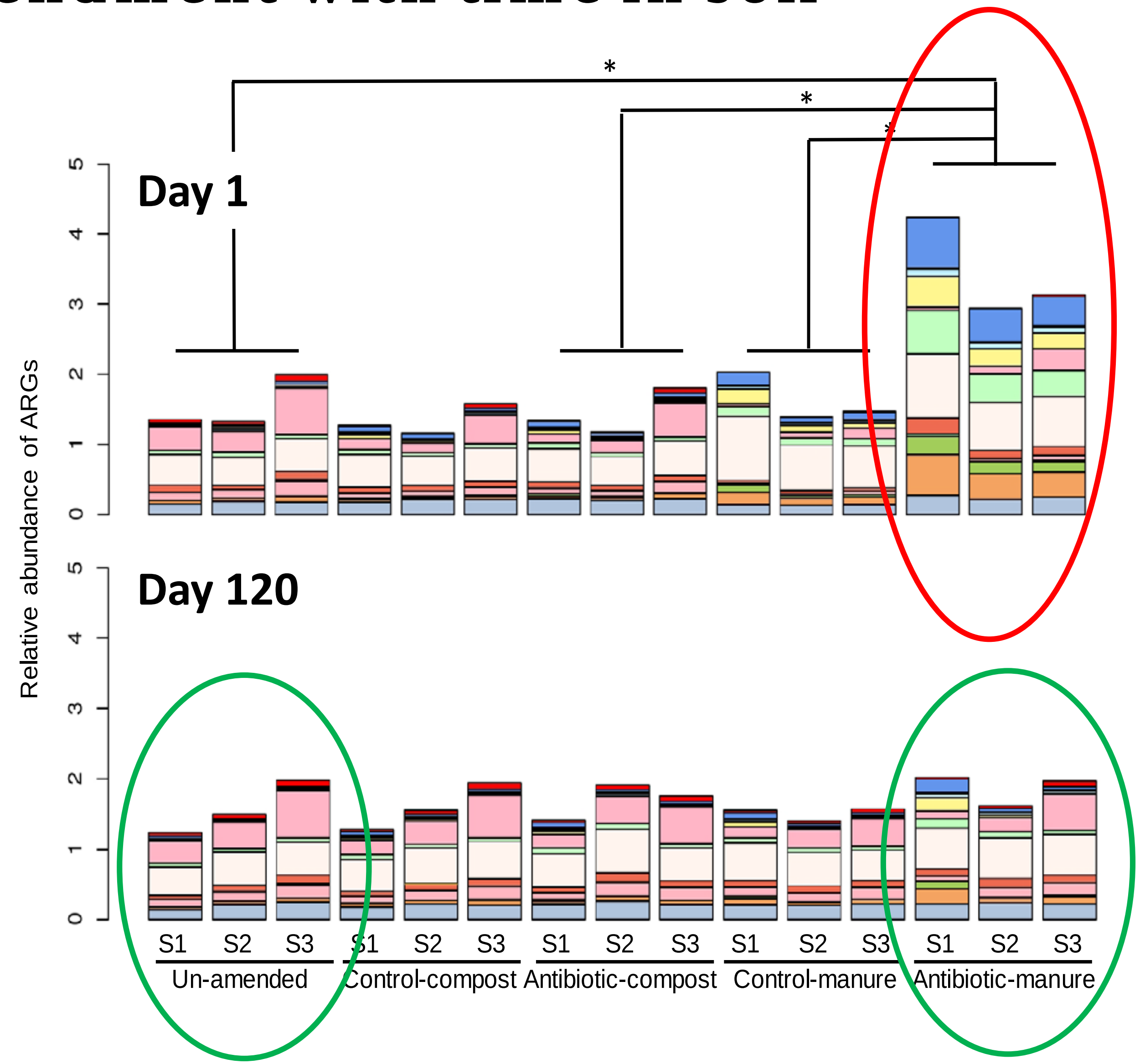
Critical Control Points for Mitigating AR from “Farm to Fork”

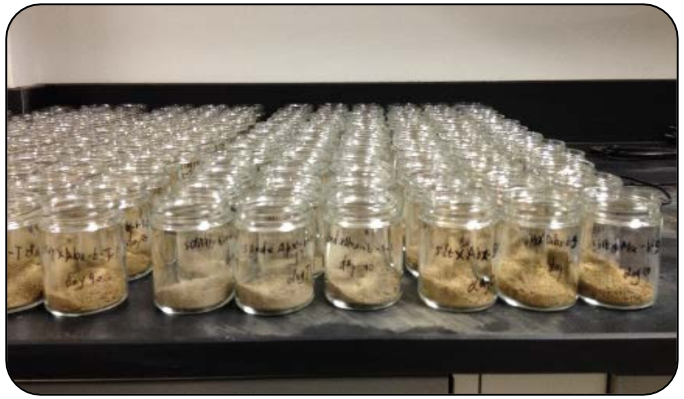


USDA NIFA

#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*

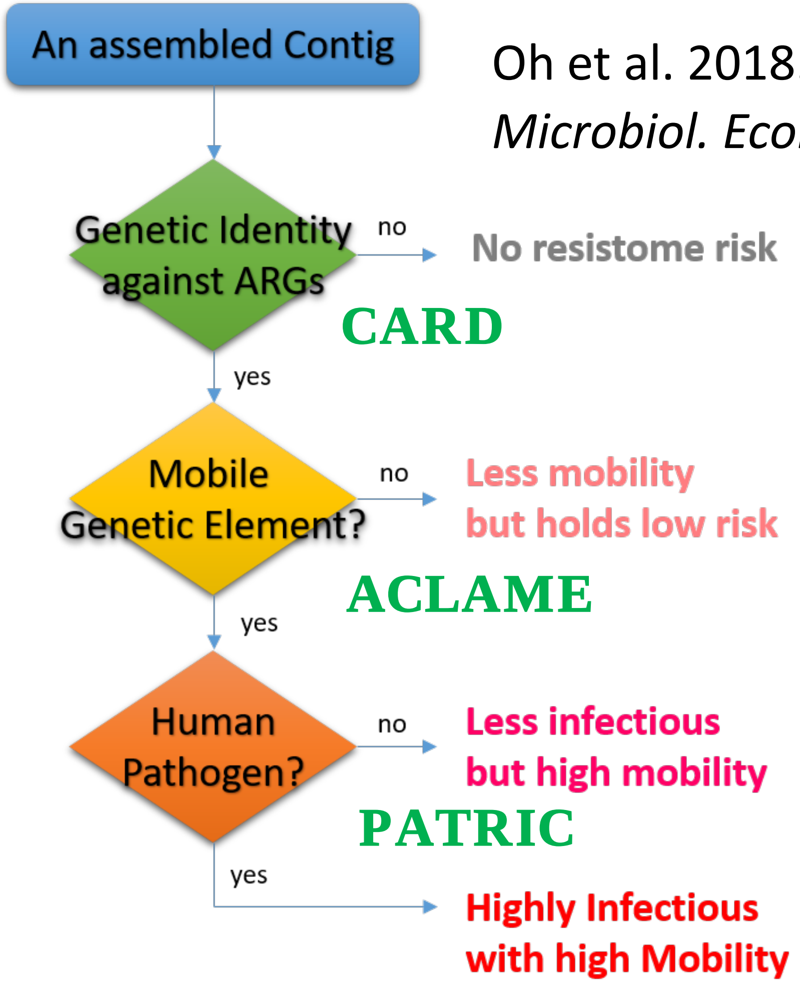
120 Days: *USDA organic guidelines- manure app-> harvest*

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

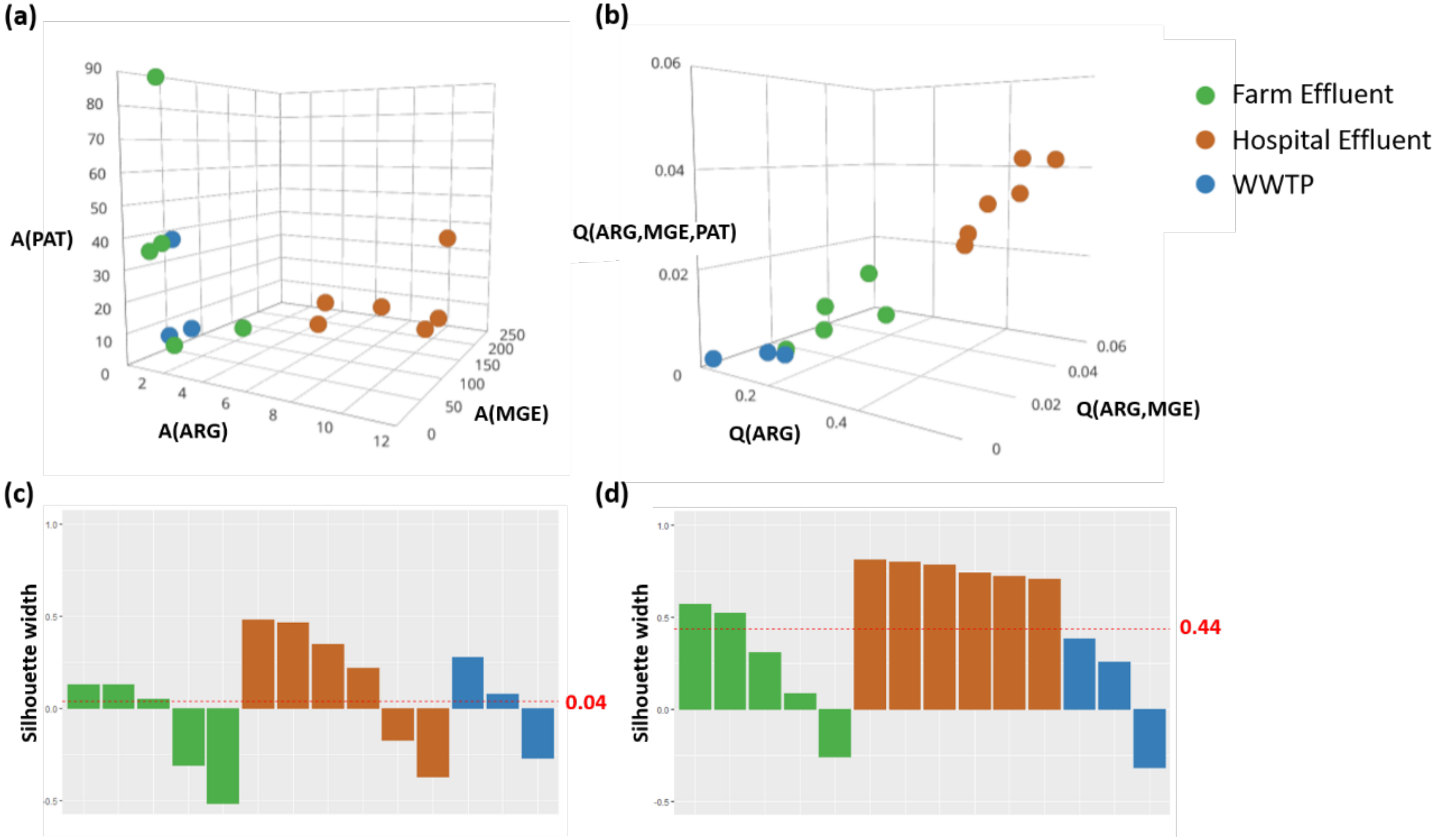
* *n=3, ANOSIM p<0.05*

Chen, Pankow, Oh *et al., in prep.*

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

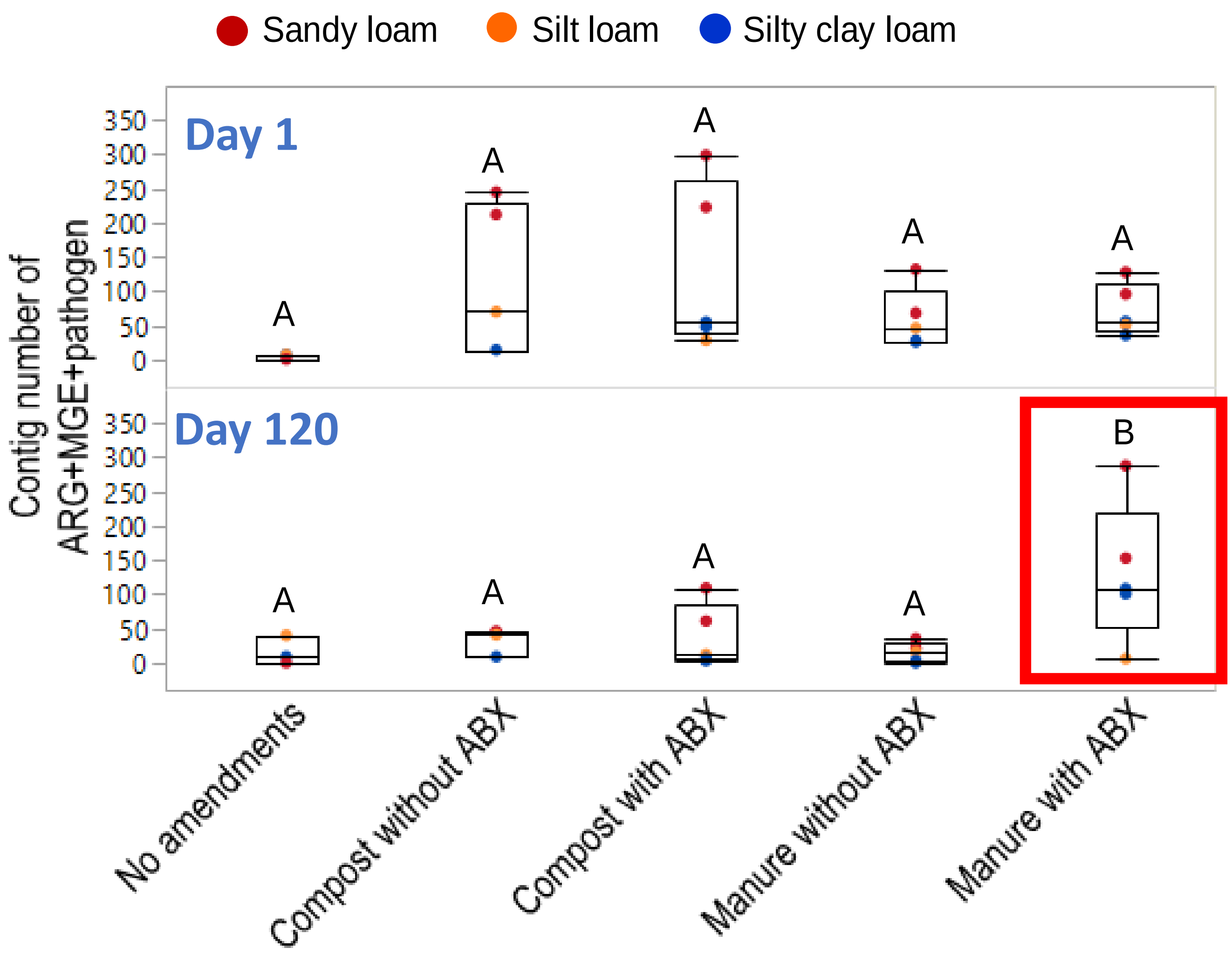


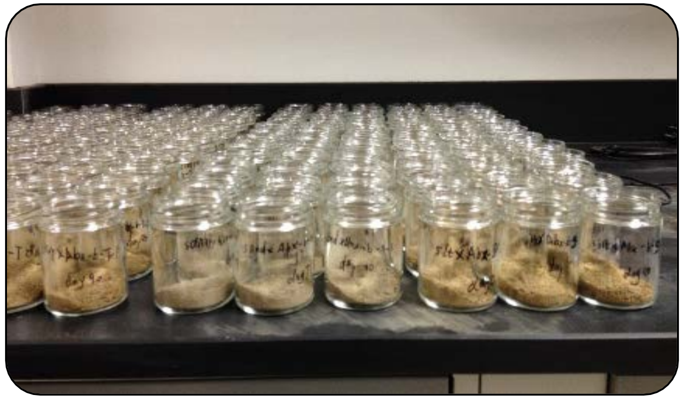
Oh et al. 2018. *FEMS Microbiol. Ecol.*



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





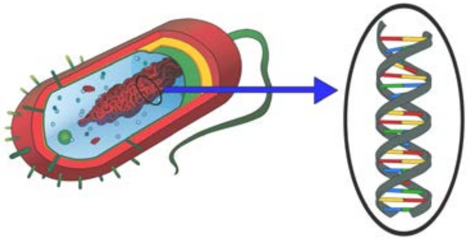
Soil Microcosms

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

Chen, Pankow, Oh *et al., in prep.*

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





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Water Reuse




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Leigh Anne Krometis

Cully Hession

Food and Ag


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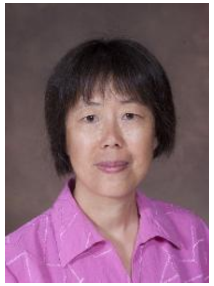
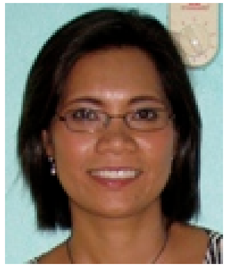
Peter Vikesland, VTech

Water Chemistry, Pipes, Wastewater, Sensors

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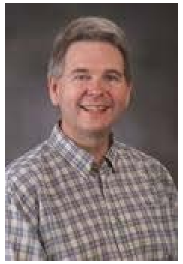
Analytical Chemistry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Bioinformatics

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www.waterrf.org

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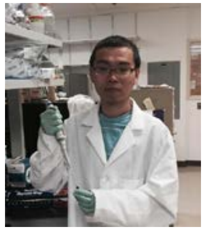




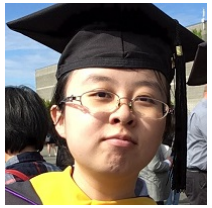

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