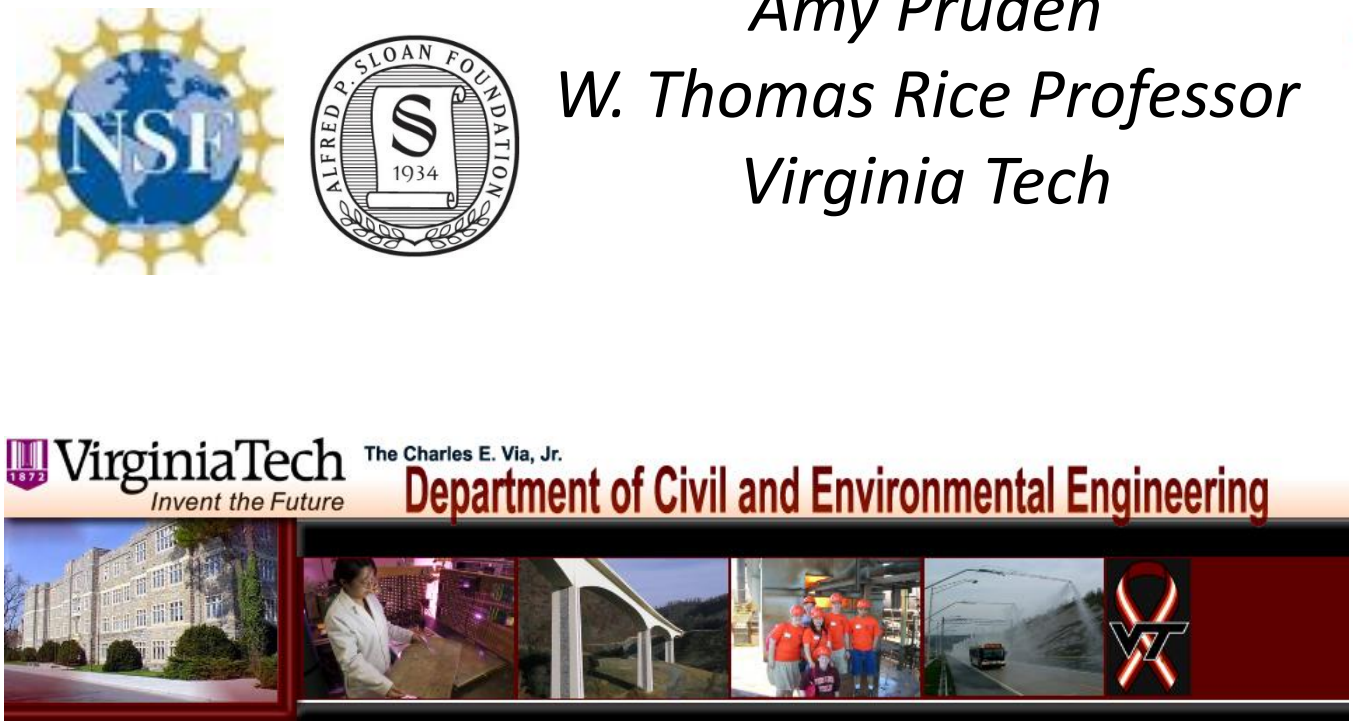
Presentation to the CEC Science Advisory Panel: *Antibiotic Resistant Bacteria (ARBs) and Antibiotic Resistance Genes (ARGs)*

Amy Pruden
W. Thomas Rice Professor
Virginia Tech



ICTAS
INSTITUTE *for* CRITICAL TECHNOLOGY *and* APPLIED SCIENCE *Virginia Tech*

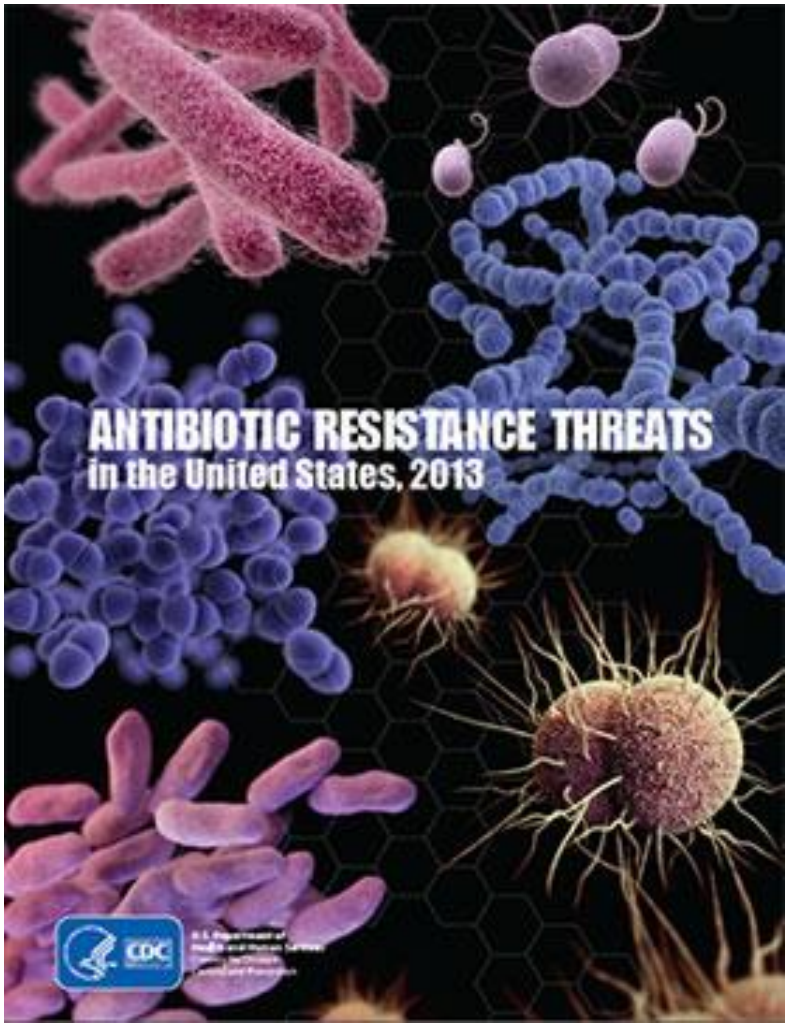
Overview

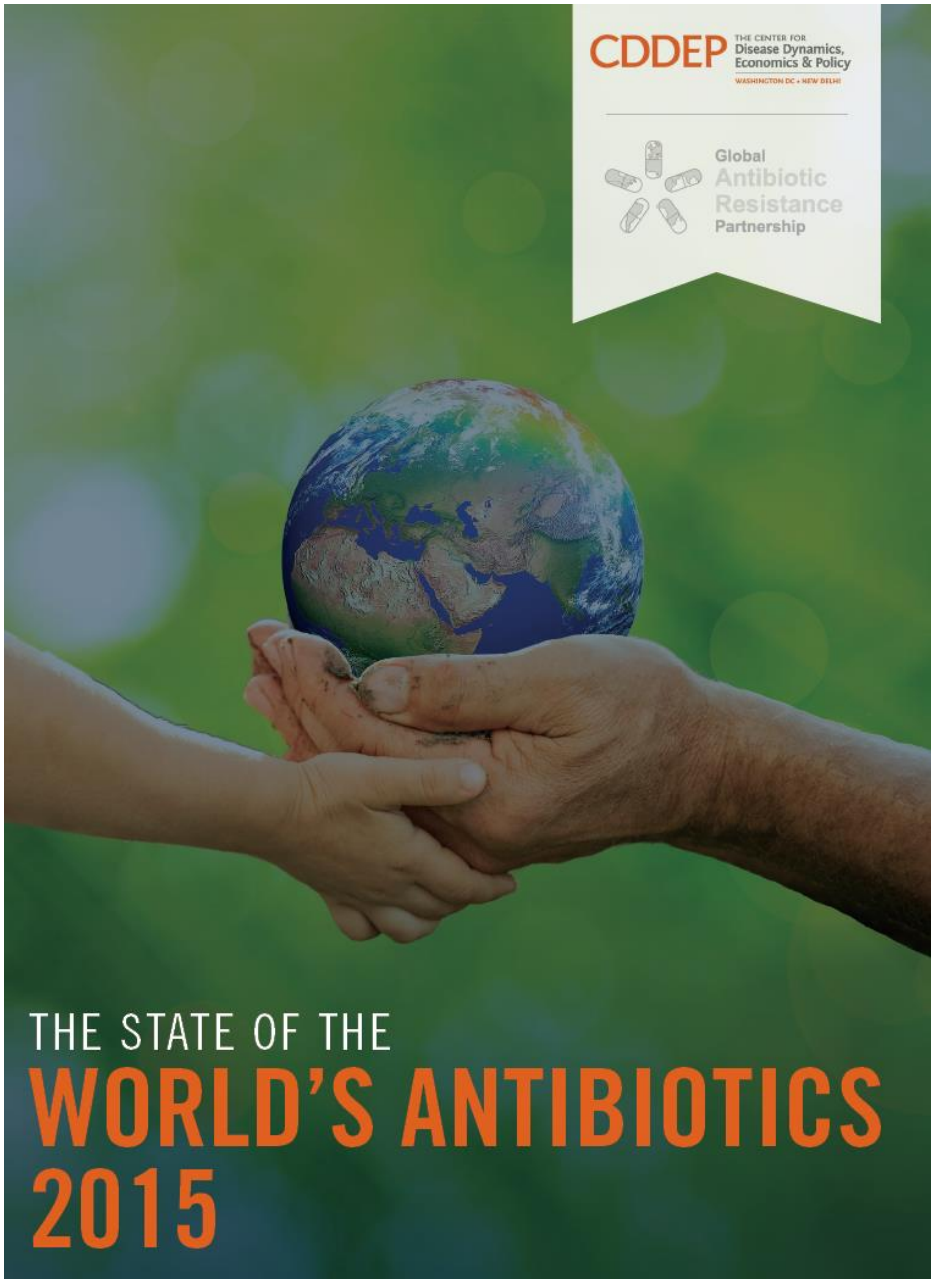
- Problem of antibiotic resistance
- Antibiotic resistance as a CEC
- Specific concerns for water reuse
- Research highlights on ARGs in recycled water
- Knowledge gaps and actionable items
- NWRI 2016 "*Evaluation of the Feasibility of Developing Uniform Water Recycling Criteria for Direct Potable Reuse*"

Antibiotic Resistance in the US

- September 2013 CDC Report:
  - 2 million Americans fall ill from antibiotic-resistant bacteria
  - At least 23,000 die as a result (many more if count complications)
  - $55 billion annual cost

"*Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community*"





- **CDDEP: US-India Global Antibiotic Resistance Partnership:**
  - In Europe, 25,000 deaths are attributable to antibiotic-resistant infections, with cost of €1.5 billion annually (EMA, ECDC 2009).
  - In India, 58,000 neonatal sepsis deaths are attributable to drug resistant infections (Laxminarayan et al. 2013)

NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

> "Without urgent, coordinated action, the world is heading towards a post-antibiotic era, in which common infections and minor injuries, which have been treatable for decades, can once again kill."
>
> — World Health Organization (WHO)

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Health

Superbugs to kill 'more than cancer' by 2050

Fergus Walsh
Medical correspondent

11 December 2014 | Health

Drug resistant E.coli bacteria are already a significant problem in Europe

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

Top Stories

Uzbek strongman's death confirmed
The Uzbek government confirms the death of President Islam Karimov, one of Asia's most authoritarian leaders, six days after he was taken to hospital.
9 minutes ago

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1 hour ago

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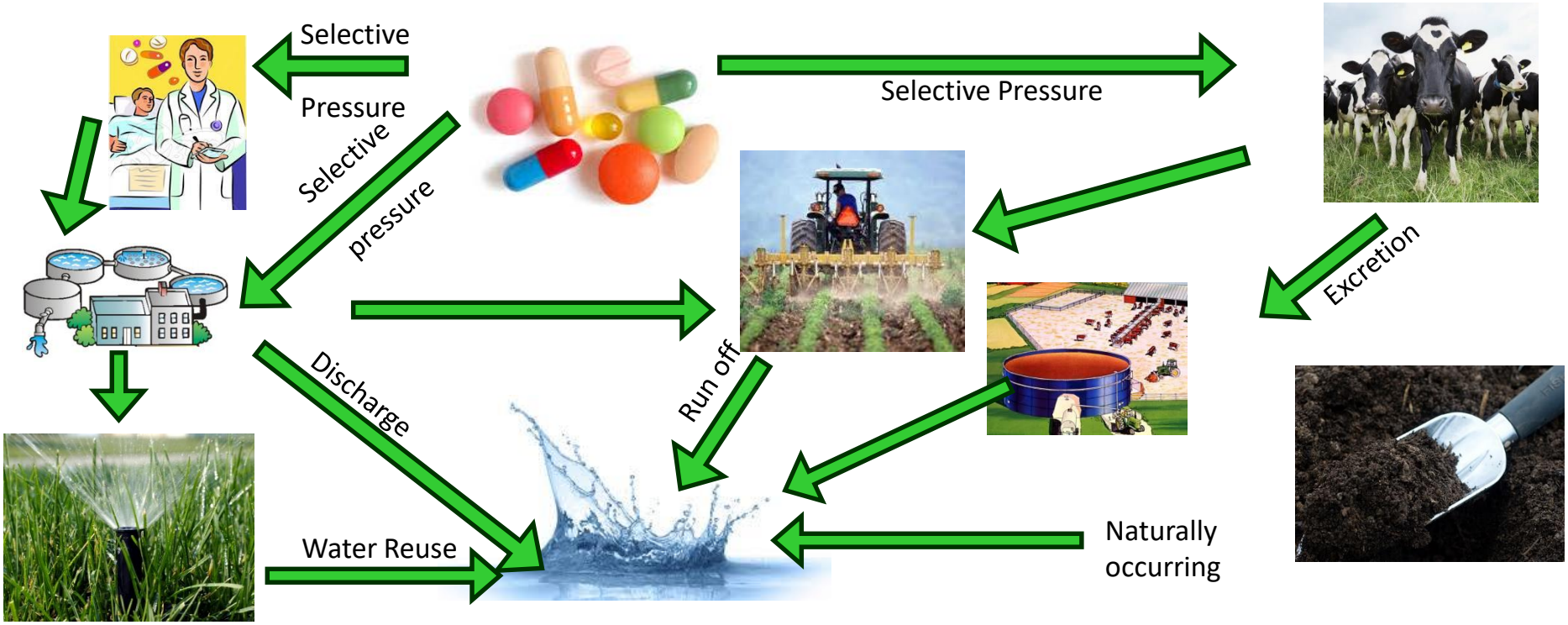
Features & Analysis

Camp life
Inside the Native American protest villages

The case for ARBs and ARGs as CECs in the Water Environment



Assaying Human Impact on ARGs in the Poudre River

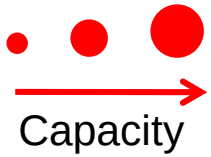
- Primary source is snowmelt
- No major tributaries
- Well-zonated
- Well-characterized in terms of antibiotics

Mapping WWTPs and AFOs

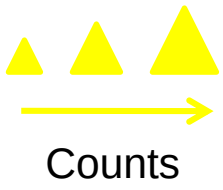
PR0a	Elephant Rock, Roosevelt National Forest
PR0b	Profile Rock, Roosevelt National Forest
PR1	Greyrock Trailhead, Roosevelt National Forest
PR2	Shields St. Bridge, Fort Collins
PR3a	Mulberry St. Bridge, Fort Collins
PR3b	Drake Reclamation Facility, Fort Collins
PR4	95th Avenue Bridge, Weld County
PR5	Greeley Municipal Airport, Greeley
SPR1	Clear Creek Confluence Park, Commerce City
SPR2	County Road 54 Bridge, Evans
SPR3	Poudre River Confluence, Kersey

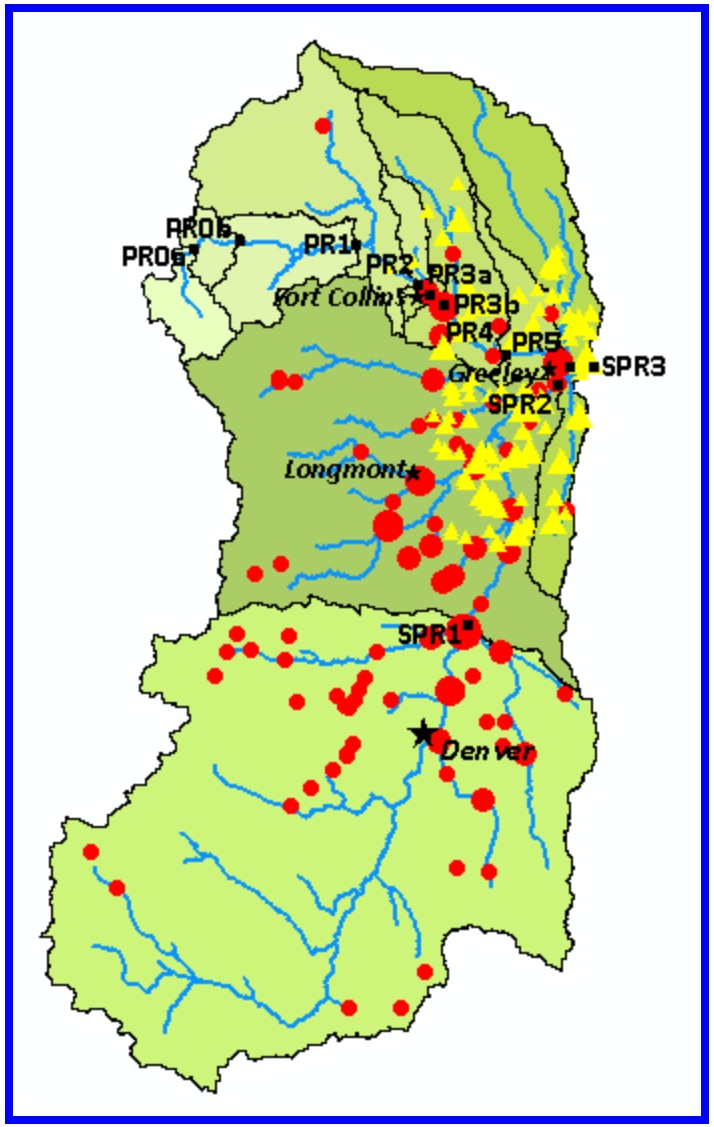
Legend

Wastewater Treatment Plants



Animal Feeding Operations

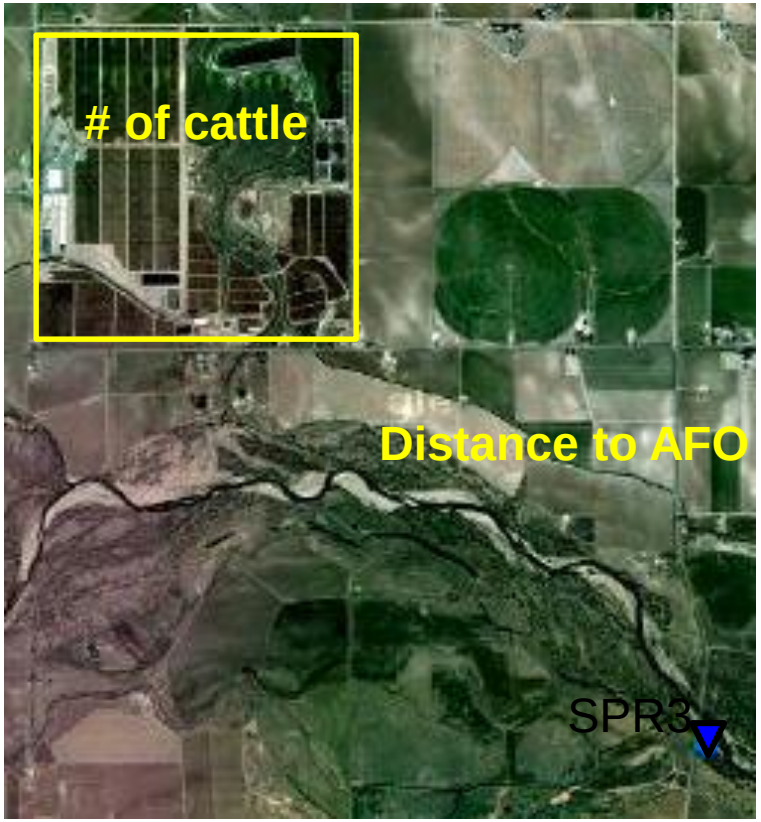




Delineating Distances from Sources to Monitoring Points

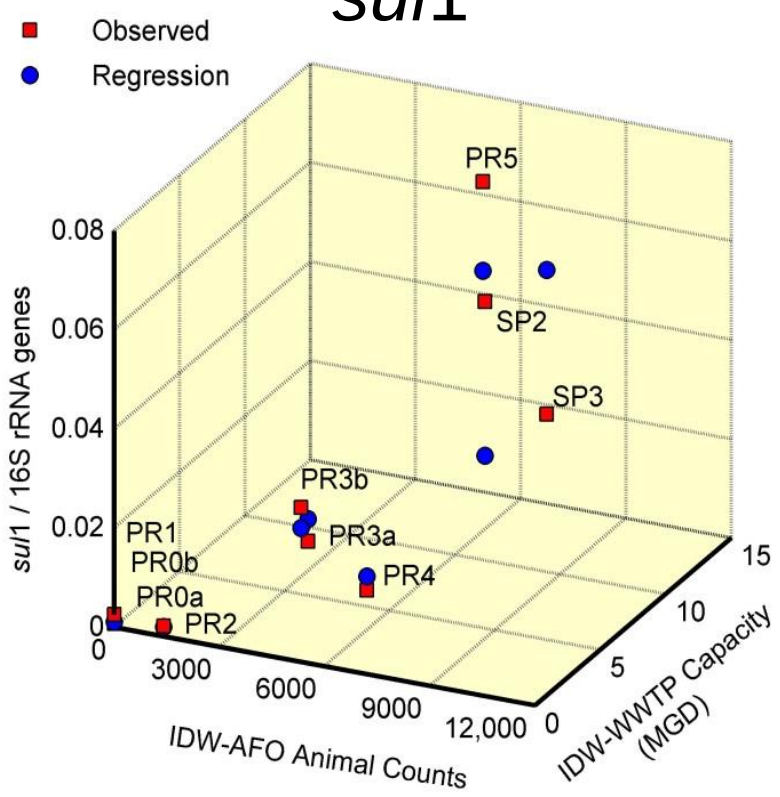
Inverse Distance Weighted (IDW) Count

$$\sum_{i=1}^{n} w_i C \quad where \; w_i = \frac{d^{-1}}{\sum_{j=1}^{n} d_j^{-1}}$$

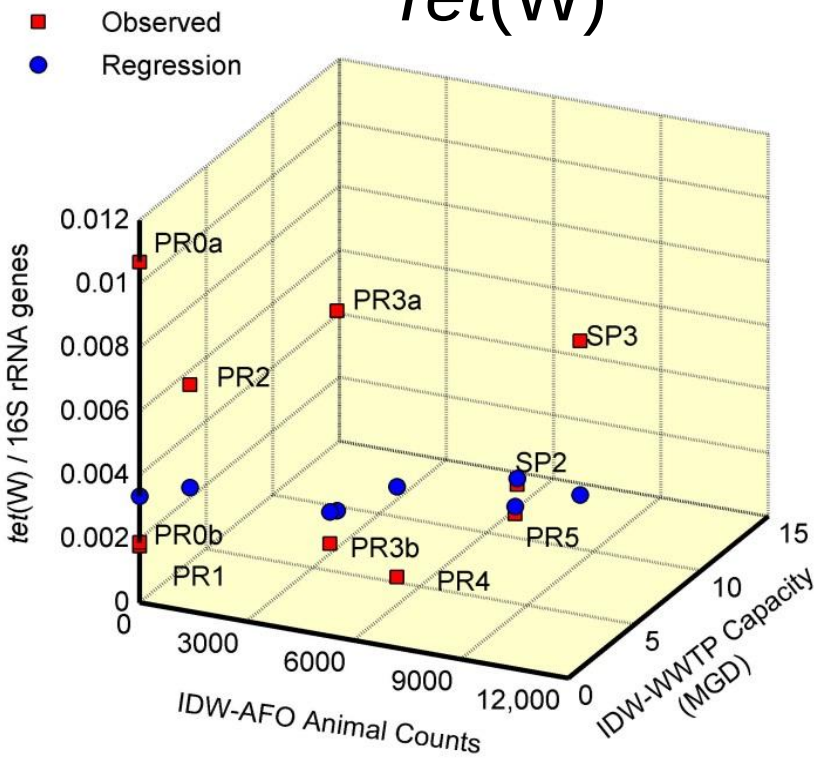


Multivariate Correlation with AFOs and WWTPs

*sul*1



Tet(W)



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

(R^2=0.23, p=0.39)

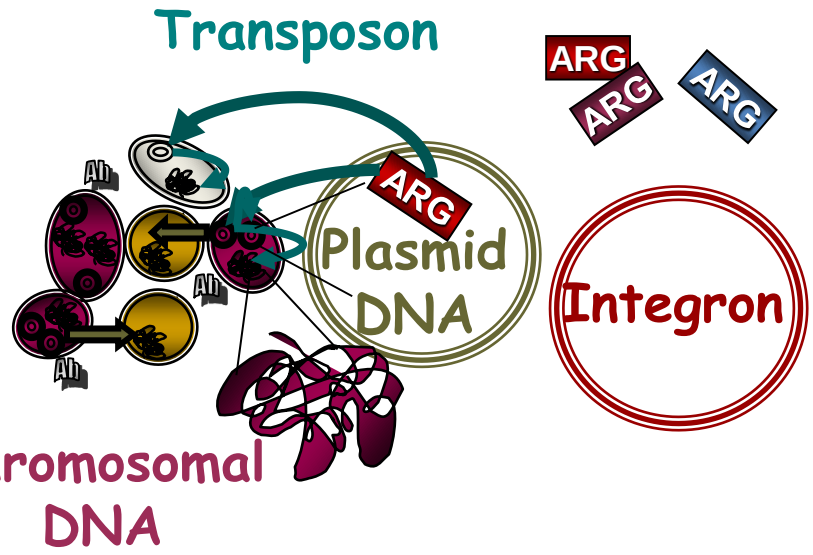
Pruden et al. 2012

Antibiotic Resistance Genes (ARGs) as CECs

Antibiotic Resistance

Mutations in chromosome

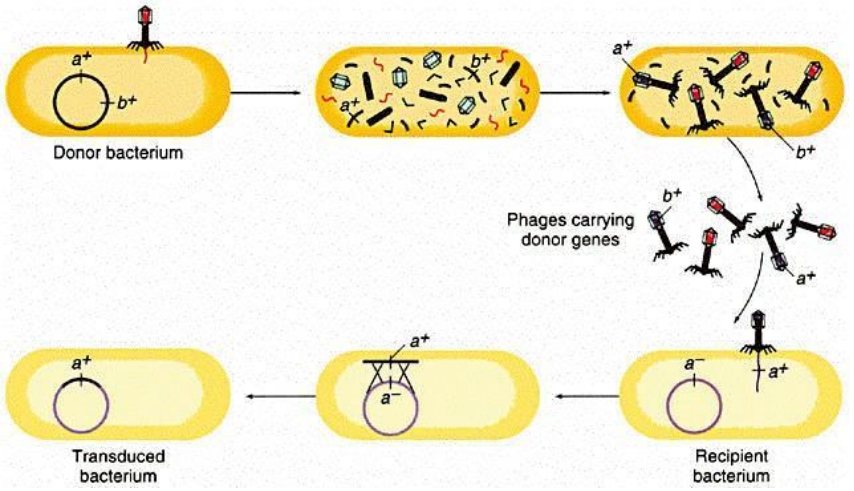
Mobile genetic elements (Antibiotic Resistance Genes or ARGs)



Horizontal Gene Transfer (HGT): Traditional approach of killing bacteria may not be sufficient- ***ideally should think about destroying ARGs*.***

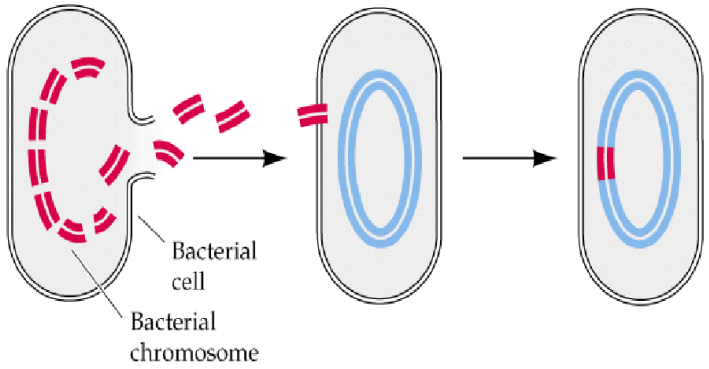
**See work of Krista Wigginton, U of MI and Michael Dodd, U WA*

How Bacteria Share Genes: Horizontal Gene Transfer



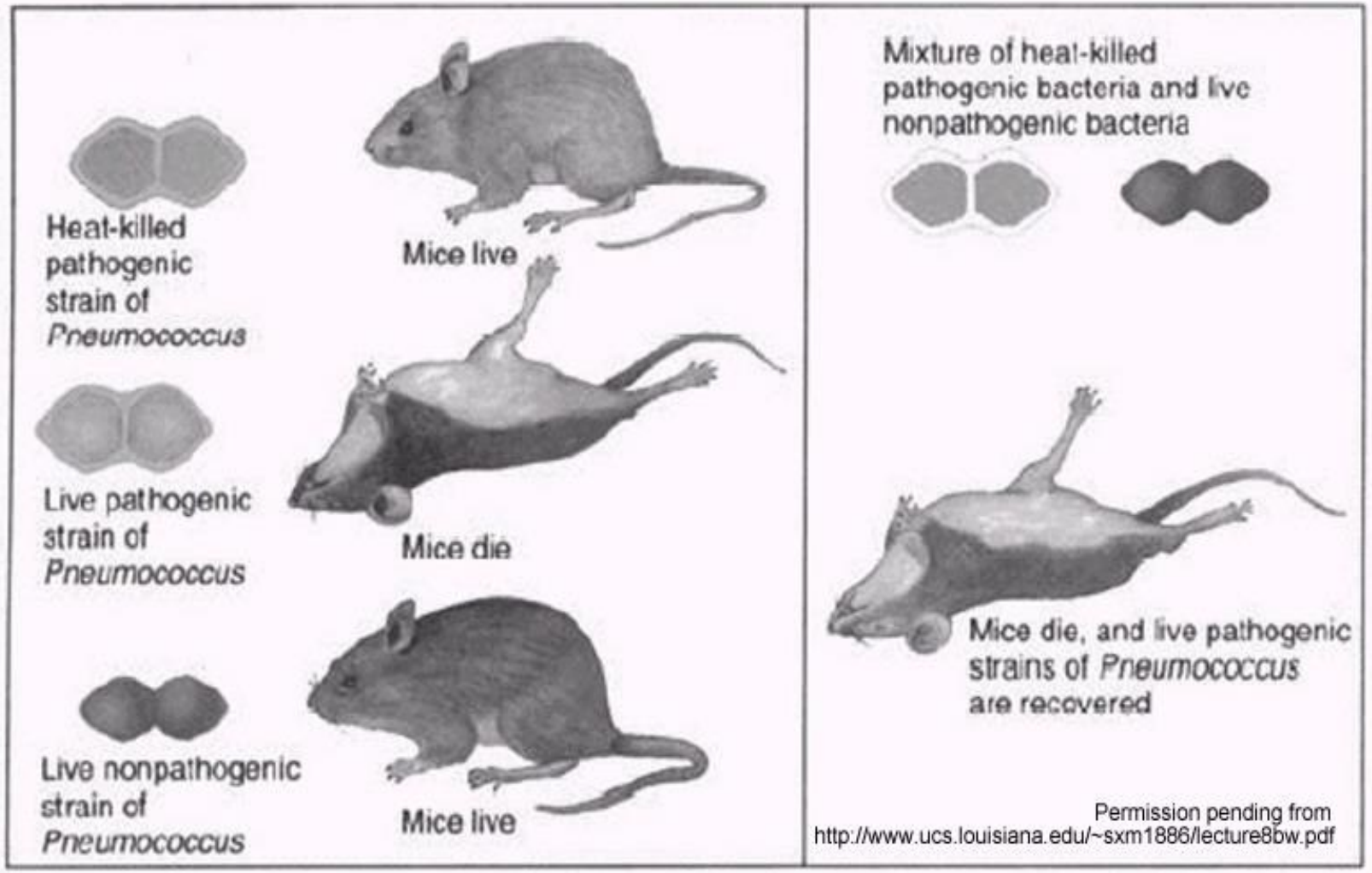
Conjugation: Bacterial "mating"

Transduction: Virus Mediated



Transformation: DNA from **dead** bacteria taken up by **live** bacteria

Deadly DNA: Griffith's Classic DNA Transformation Experiment (1928)



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NDM-1 Example

Klebsiella pneumoniae, Heather Turgeon, *Stroller Derby*

- **NDM-1** emerged out of India
- Refers to **genetic element** resistant to broad suite of antibiotics *(Yong et al. 2000)*
- Found in **multiple pathogens**:
  - *Klebsiella pneumoniae, Escherichia coli, Citrobacter freundii*, *Enterobacter cloacae,* and *Morganella morganii*
- Detected in **surface water** and **tap water** in India *(Walsh et al. 2011)* and **WWTP effluent** and **receiving waters** in China (Luo, Alvarez et al. 2014)

Proliferation of Multidrug-Resistant "Superbugs" (NDM-1 Positive) in Activated Sludge Treatment Plants

Pedro J.J. Alvarez

Clarke Prize Conference, 7 November 2014

RICE

The Clarke Prize

"One Water": Opportunities to Mitigate and Minimize Risks of ARBs and ARGs in Urban Water Systems

- Management of **Distribution System** and Other Infrastructure

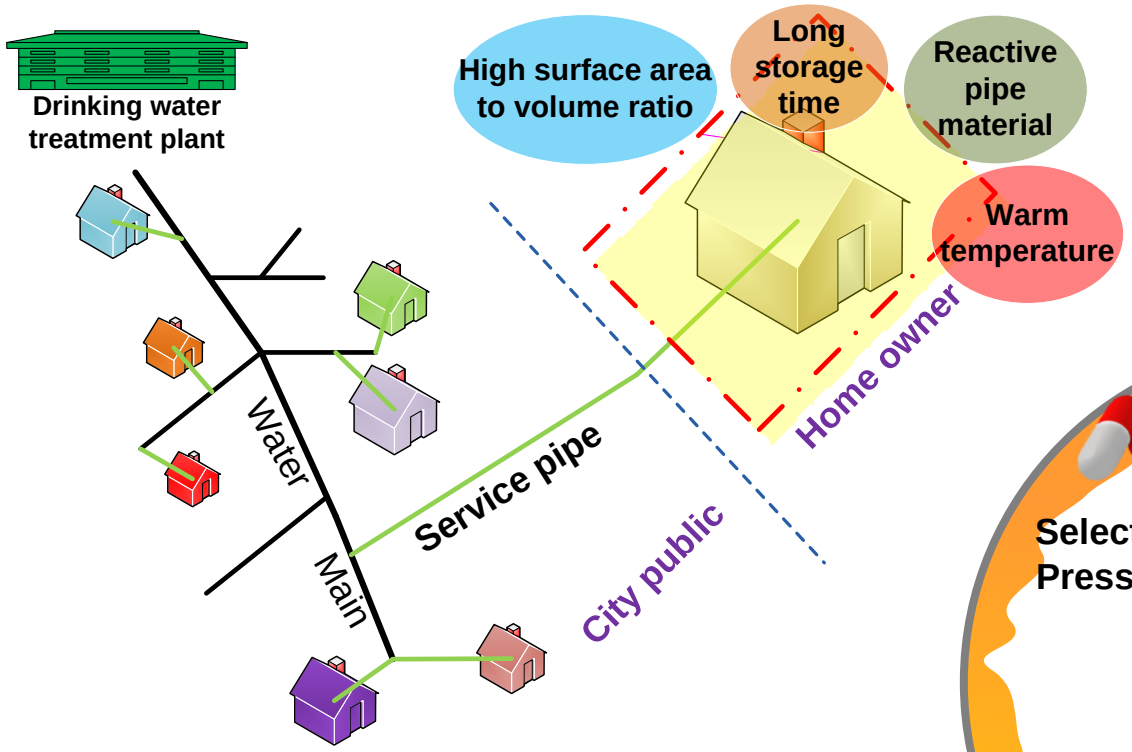
Minimize Antibiotic + ARGs Inputs *in Wastewater*

Optimize biological treatment for ***minimizing*** *ARGs and gene transfer*

UV, disinfectants, Advanced Oxidation Process ***damage*** *of Antibiotics, ARGs*

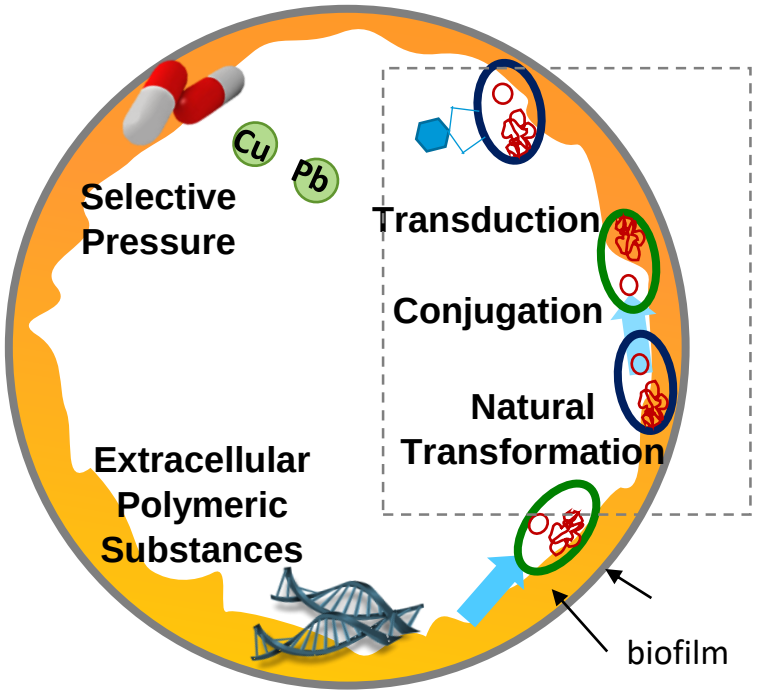
Membrane ***removal*** *of Antibiotics, ARGs*

Distribution Systems: Point of Entry vs Point of Use- Water Quality Changes in Distribution System



Artistic Credit: Hong Wang

Pipe biofilm cross-section with biofilm



Artistic Credit: Emily Garner

Special concerns for recycled water distribution?

Simulated Direct Potable Reuse (DPR) Pipes

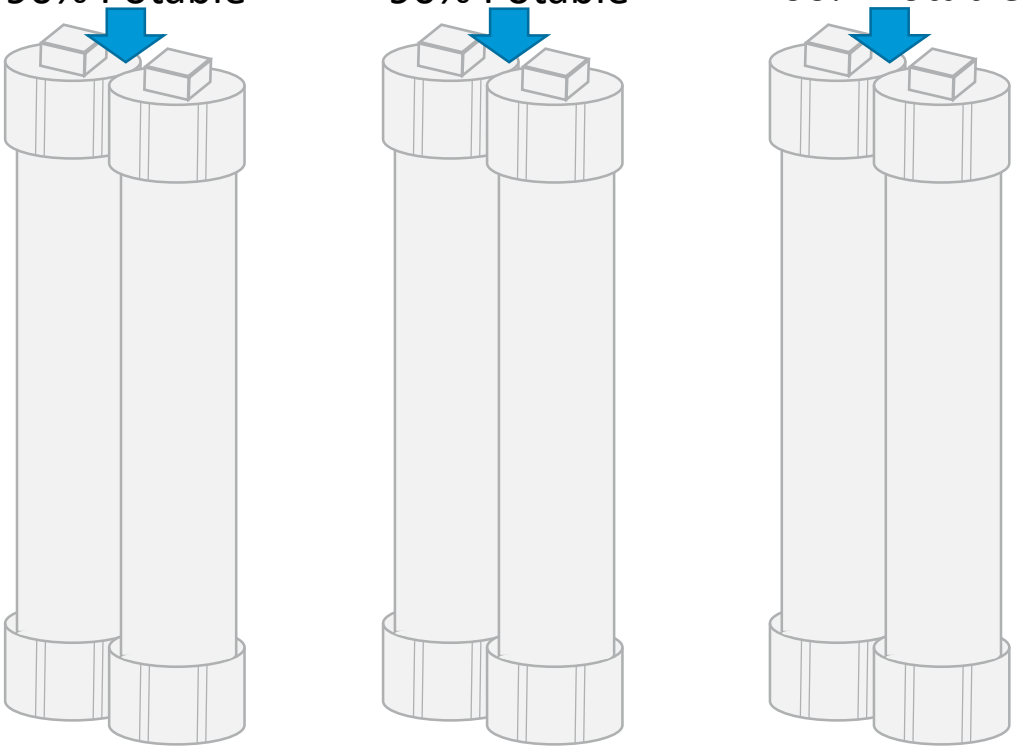
Water Research Foundation Project 4536

Emily Garner, Virginia Tech

10% DPR
90% Potable

10% DPR w/ Biofiltration
90% Potable

100% Potable



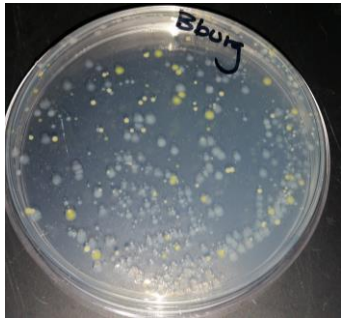
Water Change
3x / week

Sampled after
8 weeks
conditioning

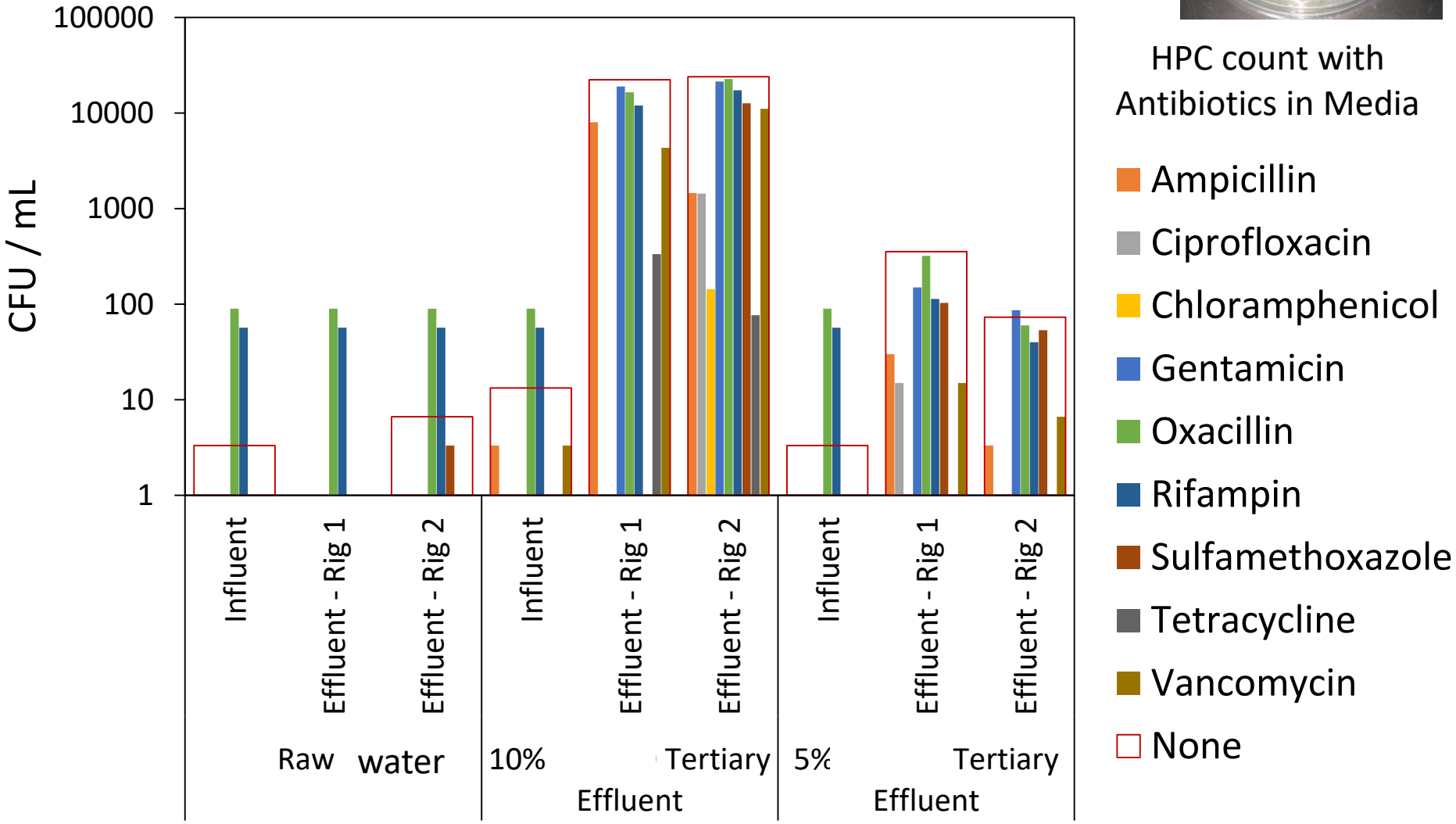
Marc Edwards, Virginia Tech

Andy Salveson, Carollo

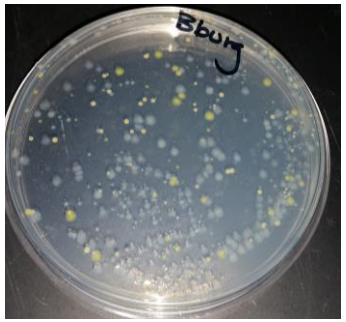
Biological N/P removal DPR source



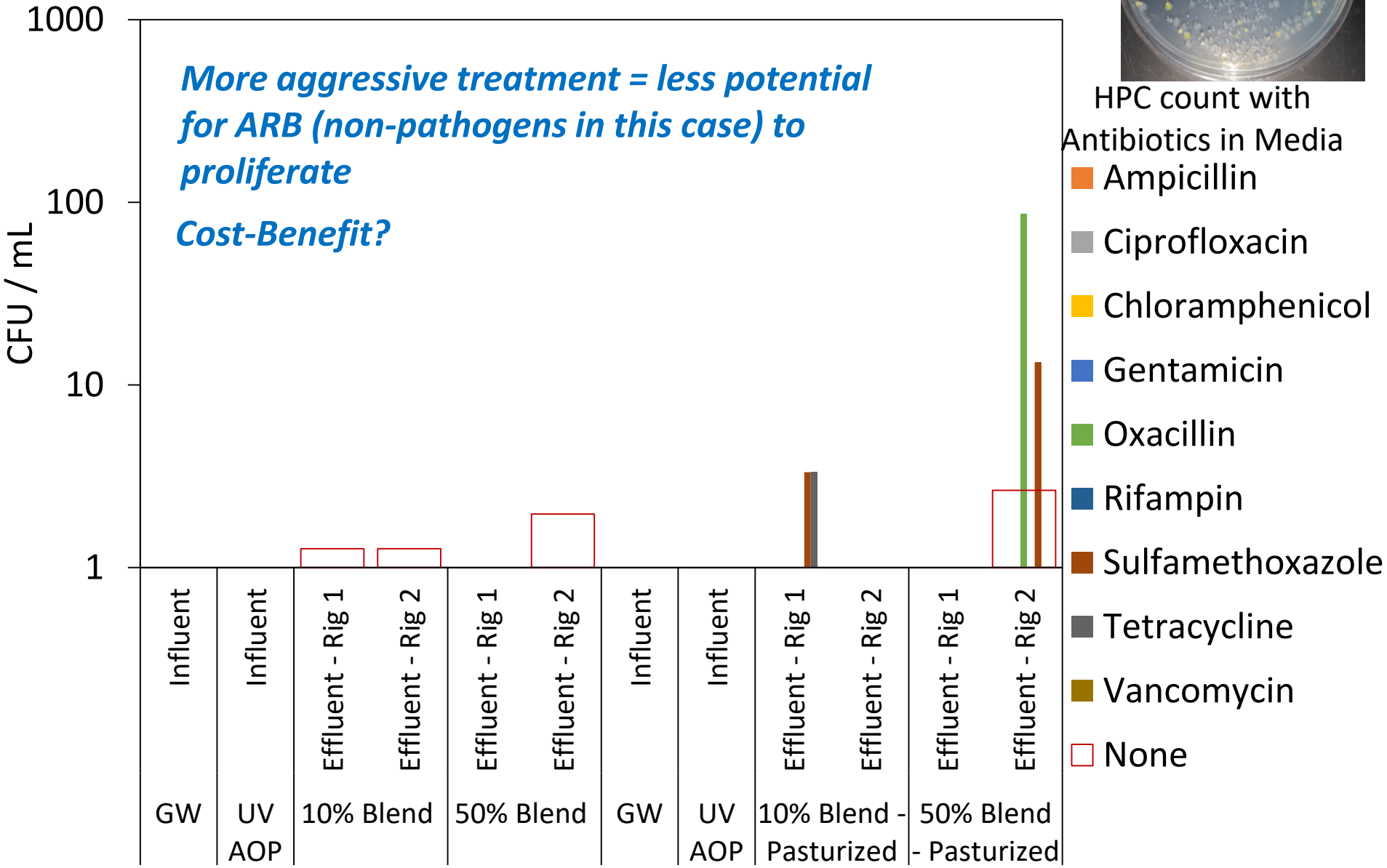
HPC count with Antibiotics in Media



Ultrafiltration, Reverse Osmosis, UV/AOP DPR Source

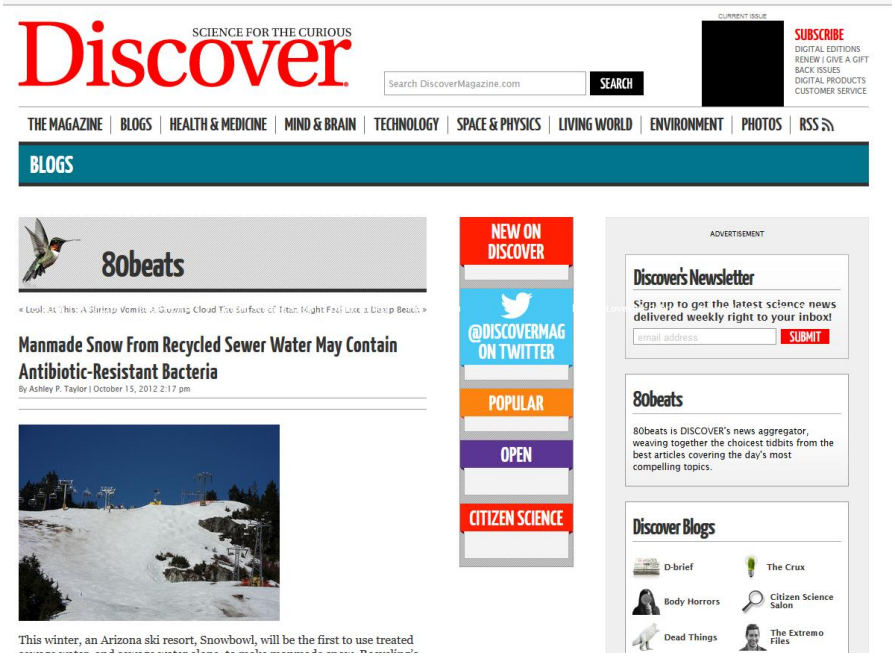


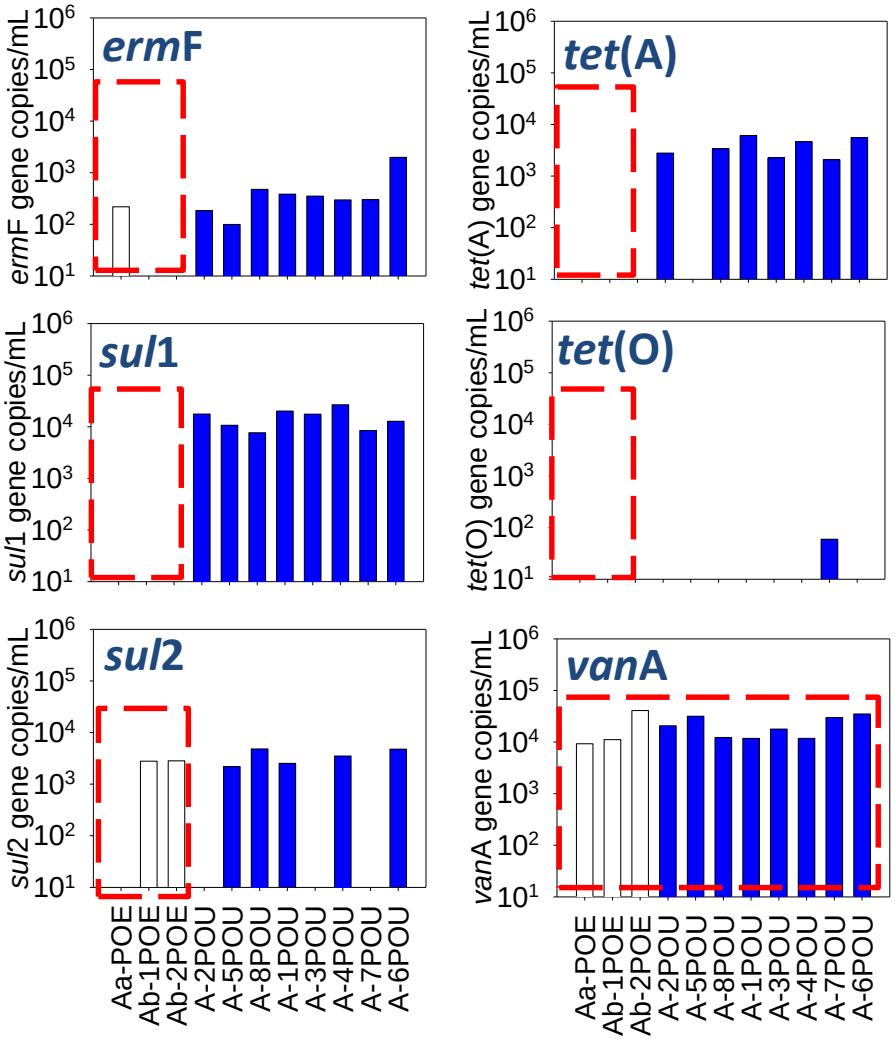
HPC count with Antibiotics in Media



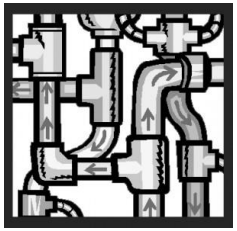
ARGs in Reclaimed Water in Flagstaff, Arizona

- Field study- non-potable reuse
- Worked with local doctor
- Shared results with city manager





Recycled Water Pipes: POE, POU



·Most ARGs detectable at the **point-of-use**, but not exiting treatment plant

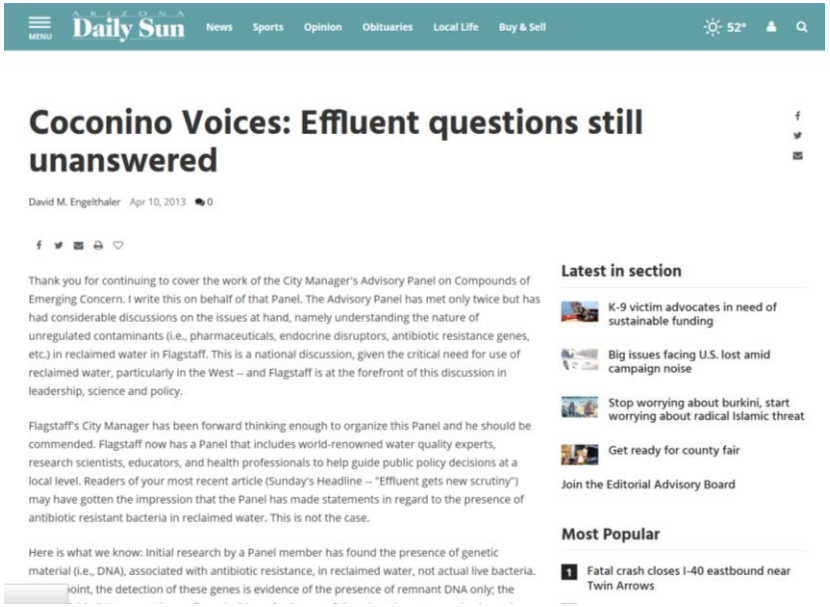
· *van*A detectable throughout

· Highlights **importance** of considering the microbiology that happens **as water flows through pipes**

Fahrenfeld et al. *Frontiers in Microbiology* 2013

Flagstaff CEC Panel Consensus Statement

- Flagstaff City Manager formed Advisory Panel on CECs
- Issued scientific panel consensus statement



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Coconino Voices: Effluent questions still unanswered

David M. Engelthaler Apr 10, 2013 0

Thank you for continuing to cover the work of the City Manager's Advisory Panel on Compounds of Emerging Concern. I write this on behalf of that Panel. The Advisory Panel has met only twice but has had considerable discussions on the issues at hand, namely understanding the nature of unregulated contaminants (i.e., pharmaceuticals, endocrine disruptors, antibiotic resistance genes, etc.) in reclaimed water in Flagstaff. This is a national discussion, given the critical need for use of reclaimed water, particularly in the West -- and Flagstaff is at the forefront of this discussion in leadership, science and policy.

Flagstaff's City Manager has been forward thinking enough to organize this Panel and he should be commended. Flagstaff now has a Panel that includes world-renowned water quality experts, research scientists, educators, and health professionals to help guide public policy decisions at a local level. Readers of your most recent article (Sunday's Headline -- "Effluent gets new scrutiny") may have gotten the impression that the Panel has made statements in regard to the presence of antibiotic resistant bacteria in reclaimed water. This is not the case.

Here is what we know: Initial research by a Panel member has found the presence of genetic material (i.e., DNA), associated with antibiotic resistance, in reclaimed water, not actual live bacteria. oint, the detection of these genes is evidence of the presence of remnant DNA only; the

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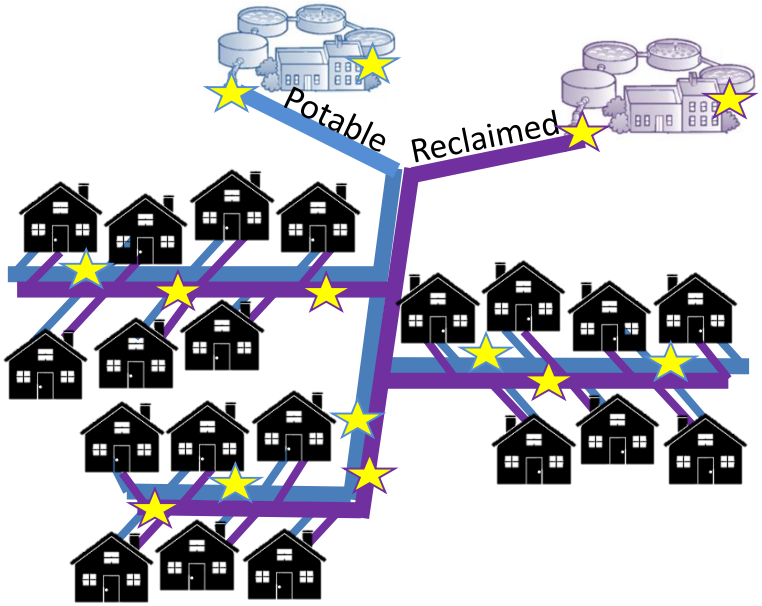
Research Questions:

- Are ARGs in recycled water different from those in corresponding potable water?
  - Diversity, types, levels…
- How do ARG profiles shift during distribution?
- What is effect of treatments/disinfectants?
- How do ARBs (*Enterococci* and *E. coli*) vs ARGs behave?

WERF

NSF

Reclaimed vs Potable Water Distribution System Survey



- Before treatment
- Point of Entry (POE)
- 4 communities

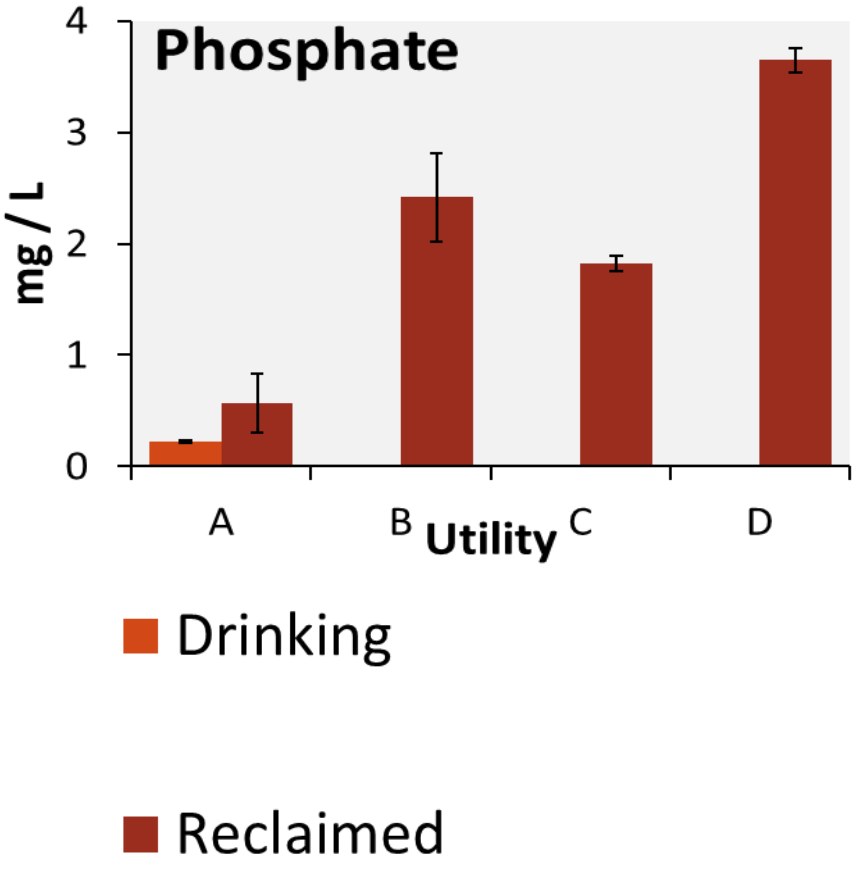
Also see work of Jjemba and LeChevallier profiling microbiology of reclaimed distribution systems

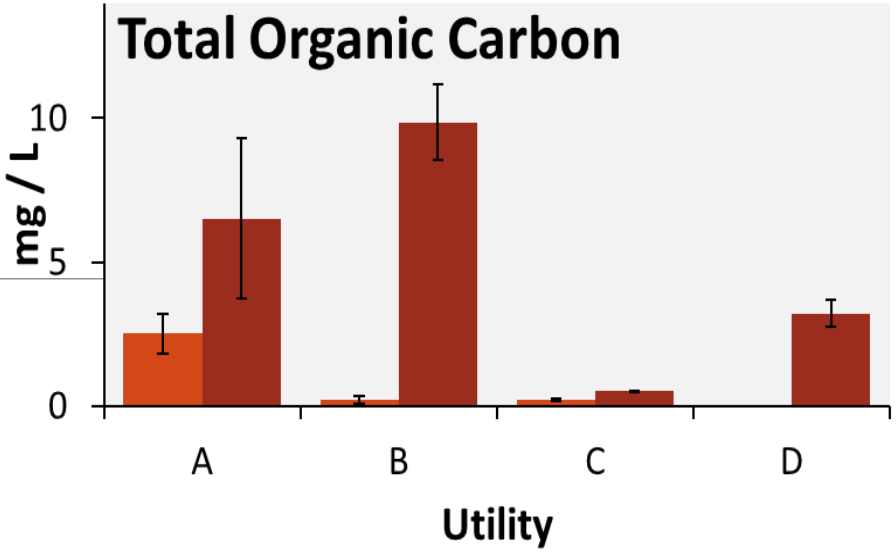
Surveyed Systems

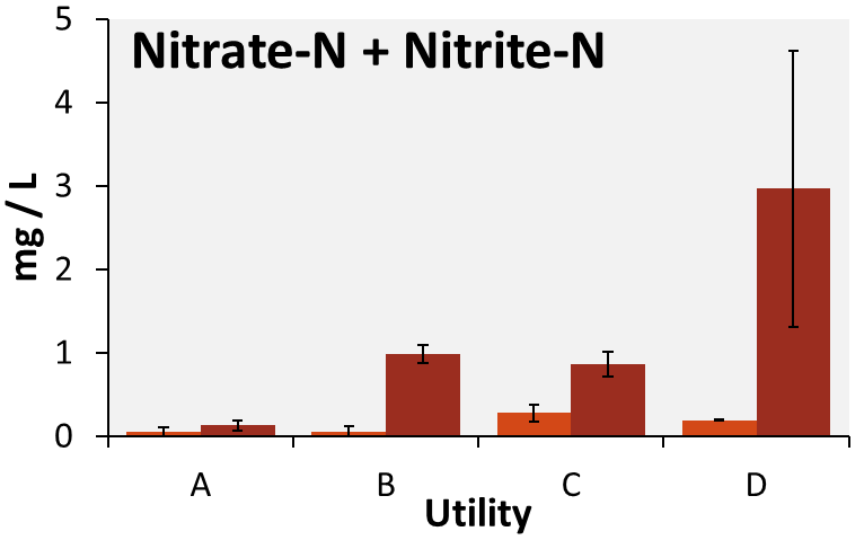
**All potable water sources are a combination of surface and groundwater*

	*POTABLE WATER	RECLAIMED WATER	
Utility	Disinfectant	Summary of Treatment	Disinfectant
A	Cl_2 ($ClNH_2$ Residual)	Plant #1 – Advanced wastewater treatment- Bardenpho Process Plant #2 – Activated sludge, secondary clarification, denitrification	Cl_2
B	Cl_2; occasional ClO_2	Plant #1 – Advanced wastewater treatment – Bardenpho Process; Plant #2 – Biofiltration, secondary sedimentation	Cl_2 UV ($ClNH_2$ Residual)
C	Cl_2	Dual media filters or membrane bioreactors	Cl_2 ($ClNH_2$ Residual)
D	Cl_2	Dual media filters	Cl_2

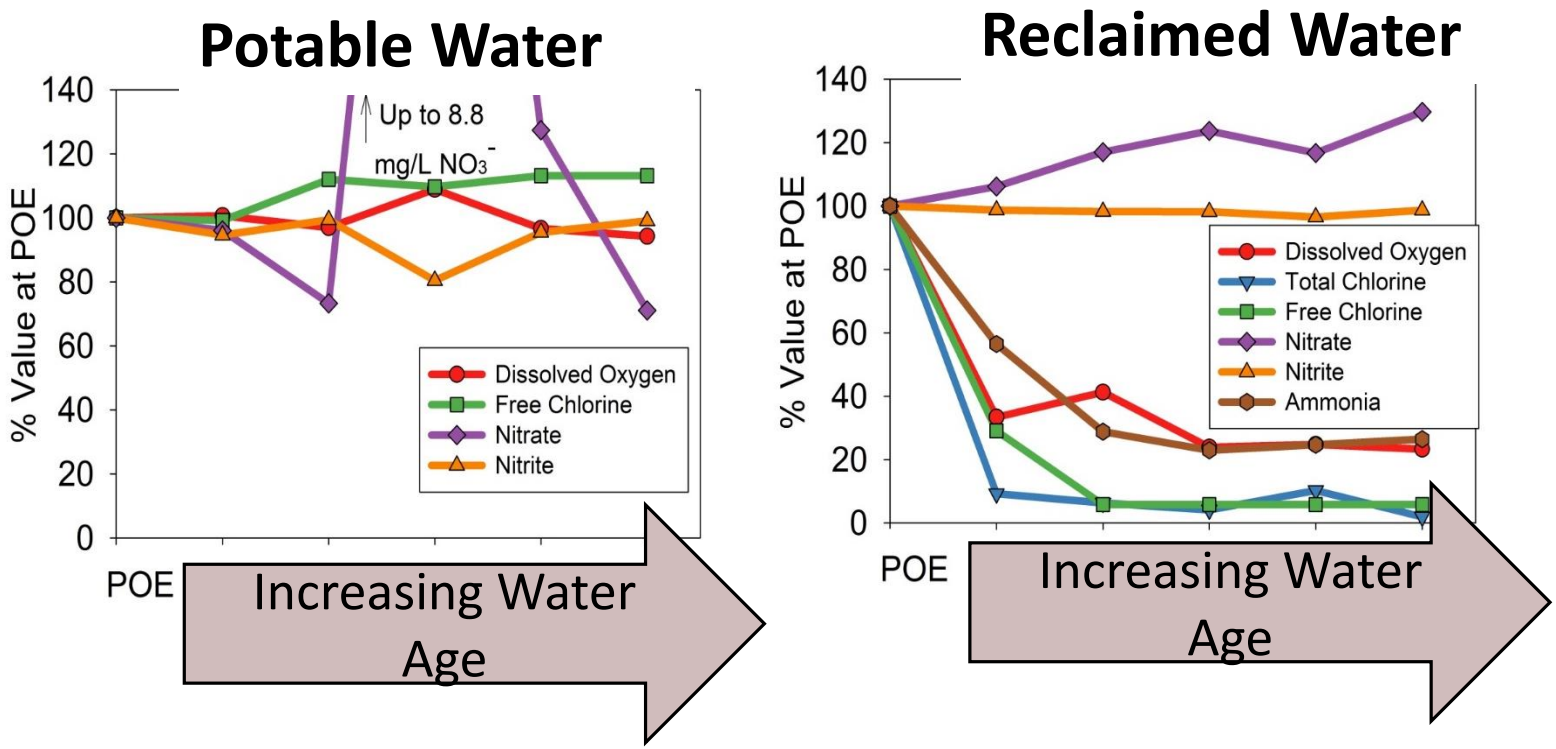
Reclaimed Water Contains more Nutrients





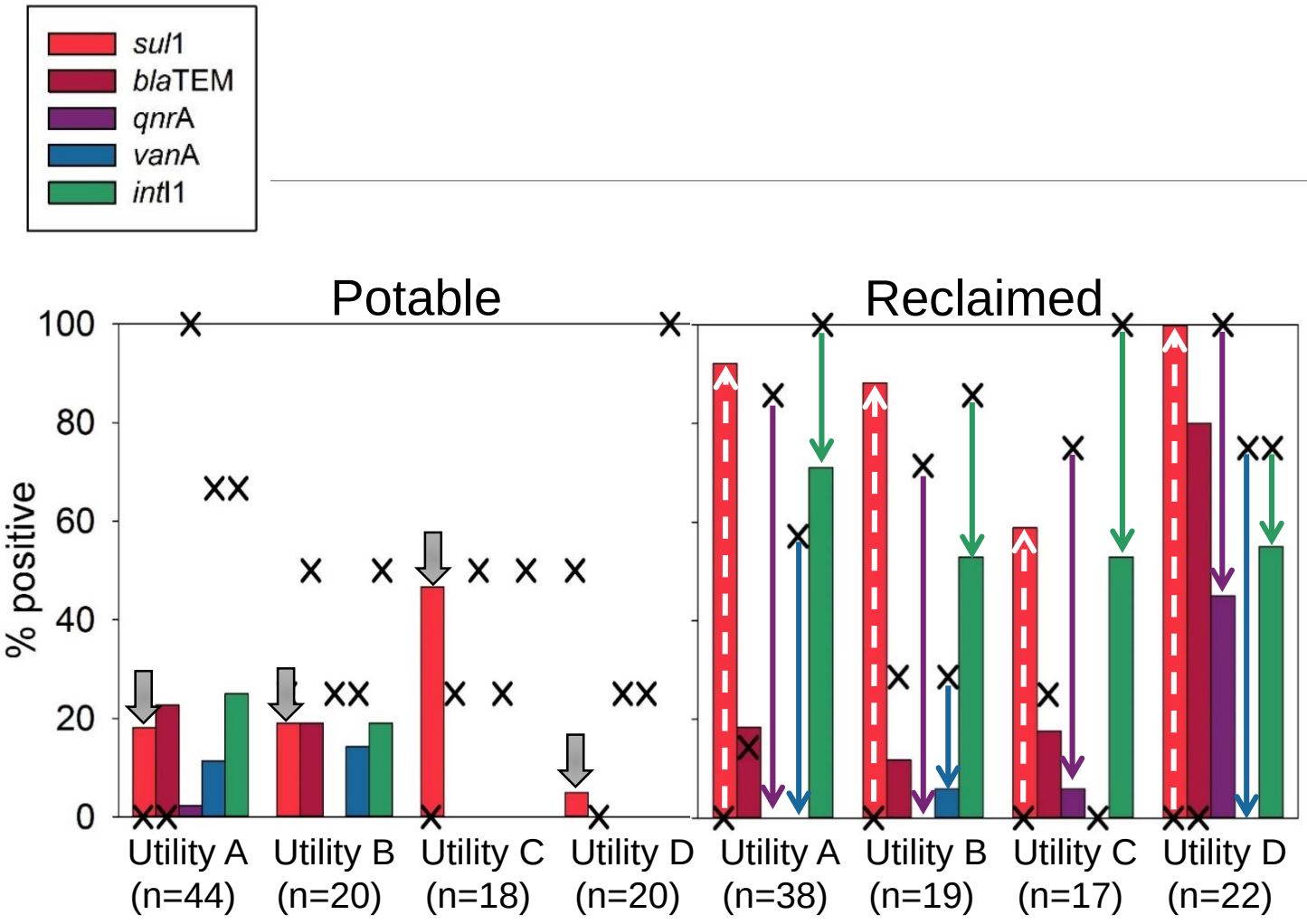


Reclaimed Water Chemistry Changes More with Increasing Water Age (*e.g., Utility C**)



**Disinfectant Residual: Cl_2*

qPCR: ARGs in Distribution System Bulk Water



Potable

*sul*1 detected at all utilities

Dissipation observed for most ARGs

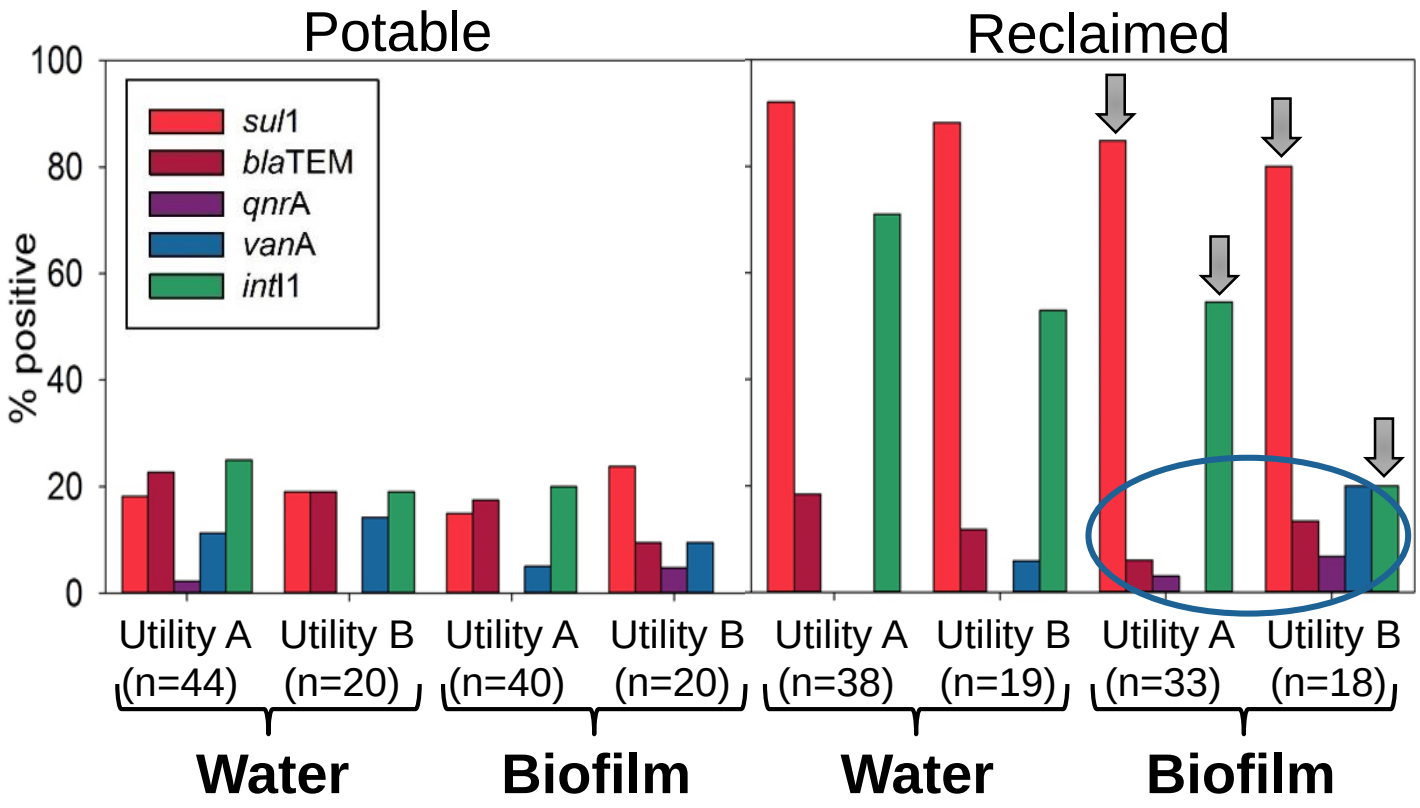
Reclaimed

*sul*1, *bla*TEM, and *int*I1 detected at all utilities

Dissipation observed for *qnr*A, *van*A, *int*I1

Increase in detection for *sul*1

qPCR: ARGs in Bulk Water vs. Biofilm

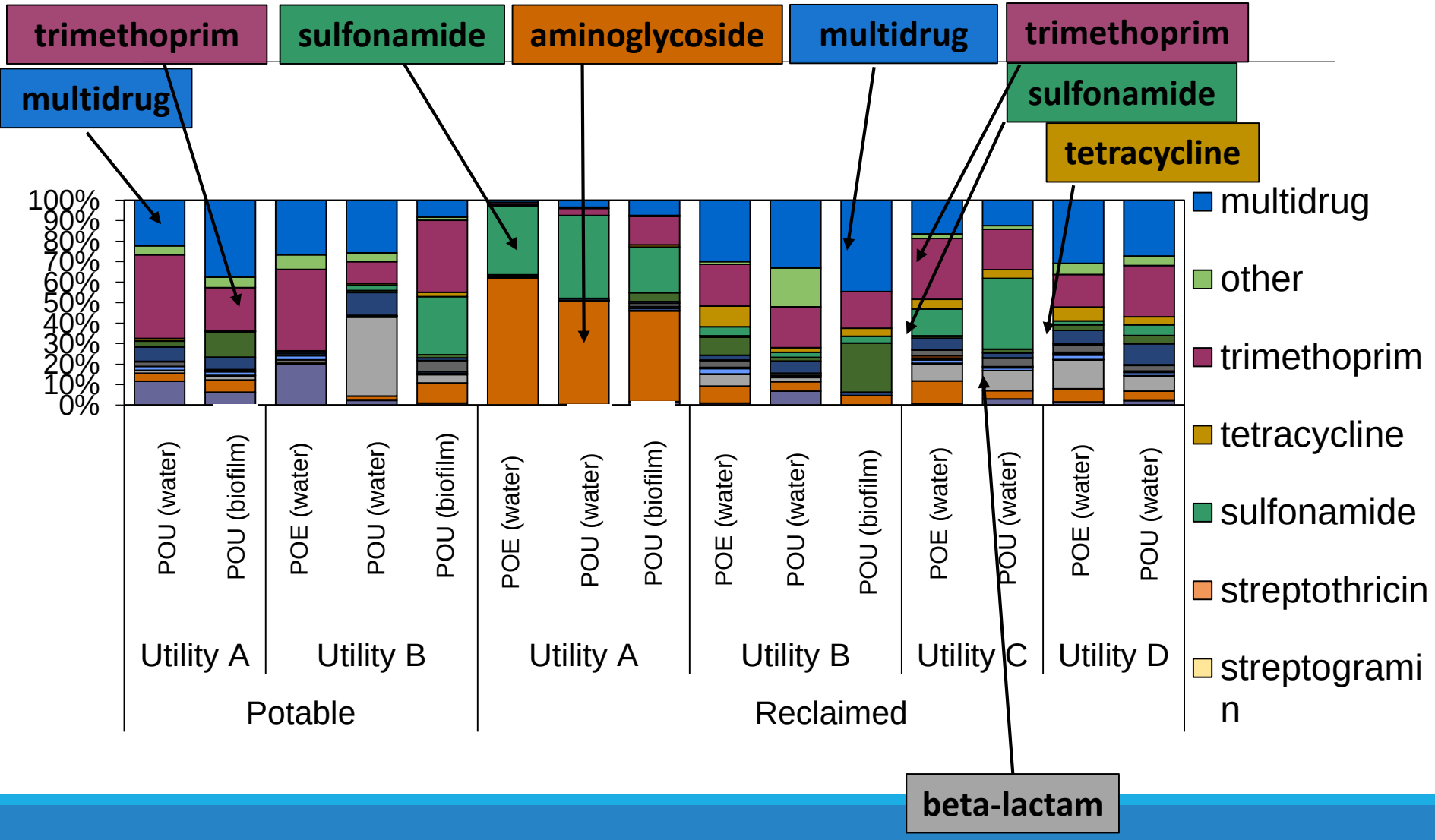


Potable
Similar detection pattern for bulk water and biofilm

Reclaimed
More ARG targets detected in biofilm than bulk water – Biofilms important sampling location?

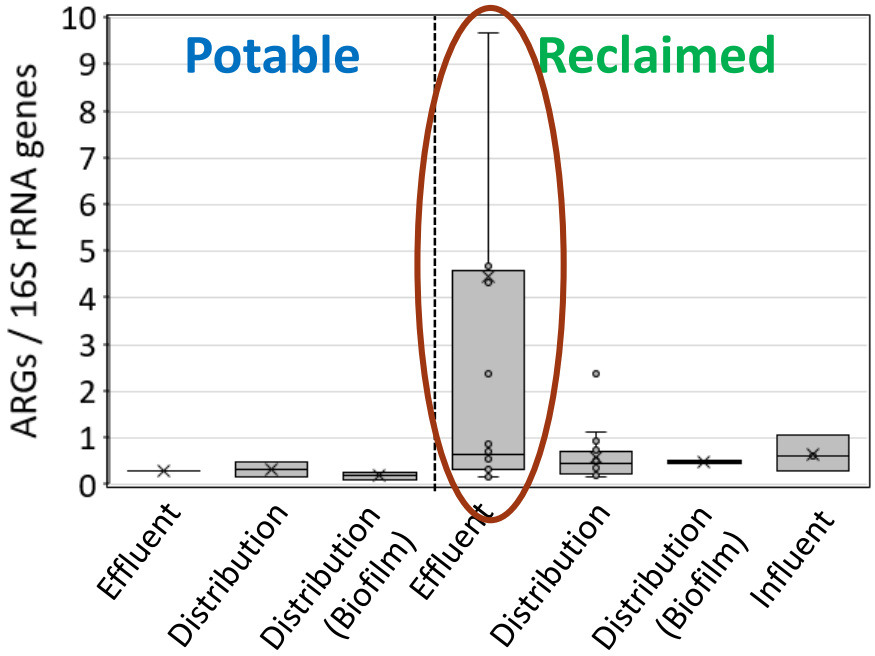
*sul*1, *intI*1 more frequently detected in reclaimed biofilms than potable

Metagenomics: Distinct "Resistome" in Reclaimed vs Potable Water Systems

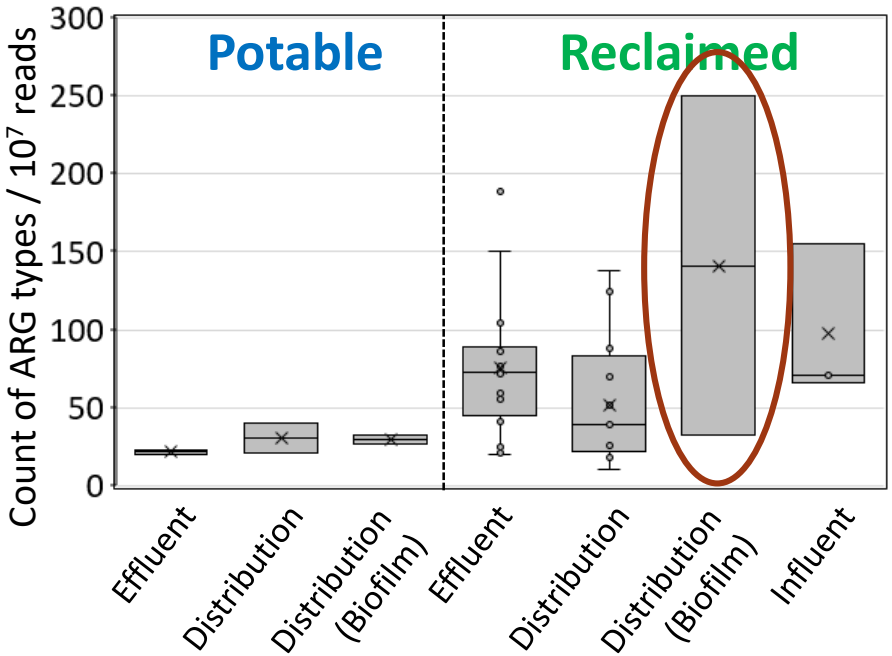


Metagenomics: ARGs More Diverse in Reclaimed Water (*across systems, time points*)

Relative Abundance of total ARGs

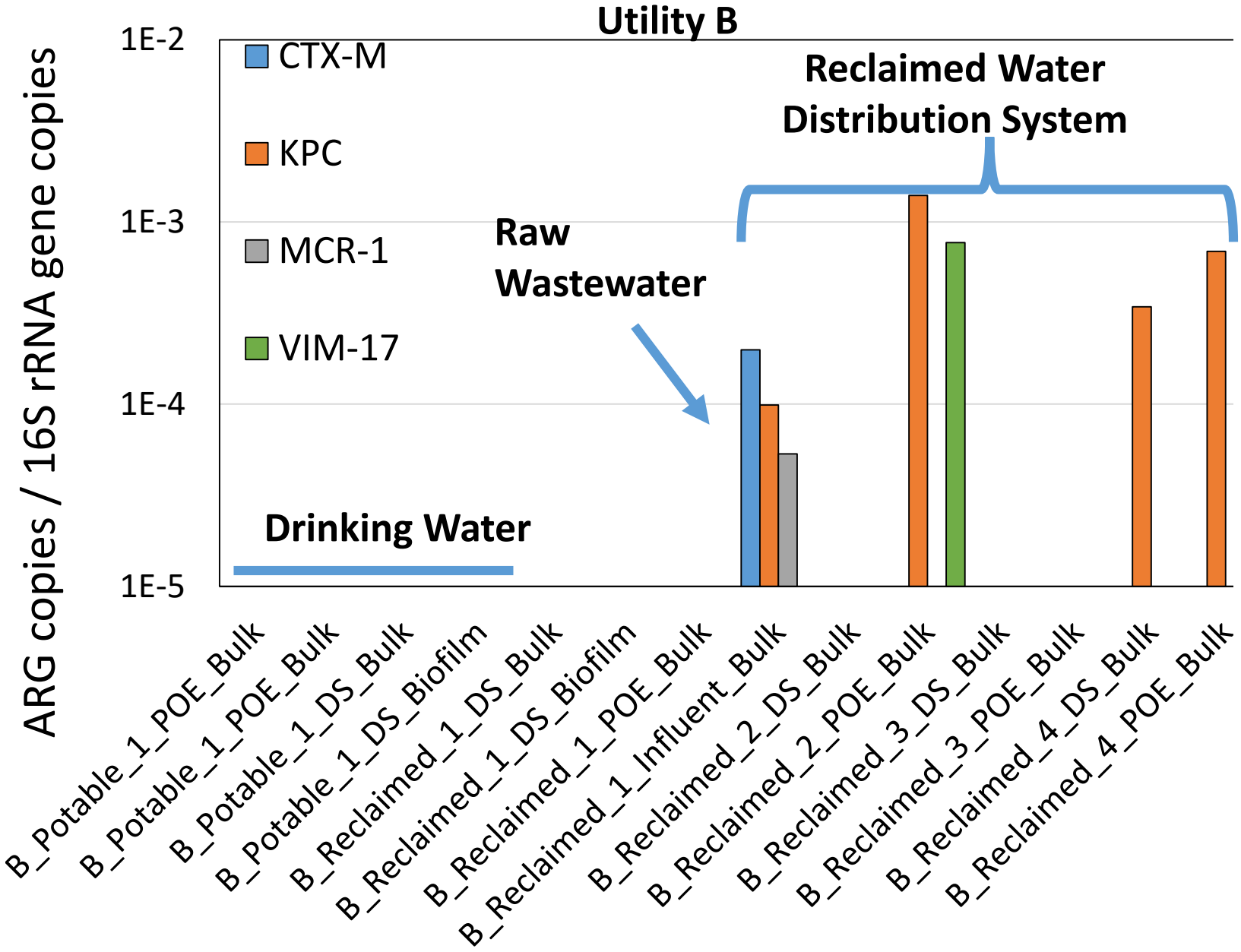


"Diversity" of ARGs



Unknown at presence if or how relative abundance of ARGs or diversity of ARGs translates to actual risk

Metagenomics (DNA): Clinically-relevant ARGs



***CTX*-M-** ESBL

KPC- *Klebsiella* pneumonia carbapenem resistance

***mcr*-1-** A colostin resistance

***vim*-17-** Verona integrin-encoded metallo-beta lactamase carbapenem resistance

Knowledge Gaps and Actionable Items

Monitoring

Monitoring is the 1st step and is widely recommended by the WHO, CDDEP, ASM, and others- But *WHAT* to Monitor?

- Antibiotics?
- Pathogenic ARBs (pARBs)?
  - e.g. Amy Sapkota's work on MRSA and VRE in reclaimed water
- Environmental ARBs (eARBs)?
  - Represent environmental reservoirs
  - MICs not well-defined
  - Culture methods only capture a fraction-
  - Target Gram + (Gerrity, McLain, and Rock)
- ARGs?

WHERE to monitor, and to *WHAT* end points?

-Post treatment as well as point-of-use

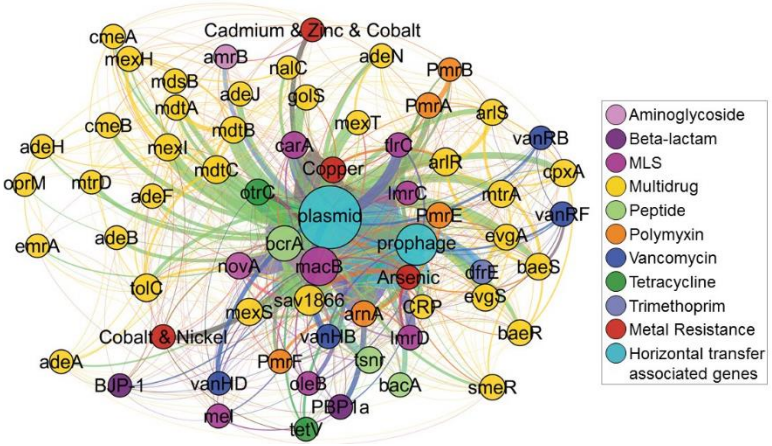
Methods- qPCR of ARGs

"Indicator" ARG Concept

- **NORMAN COST Action, StARE:** European Effort for Standardization of Methods for Monitoring ARGs in WWTPs
- Current Targets for qPCR:
  - *intl1, sul1, sul2, blaCTX-M, blaTEM, qnrS (of group p1) and ermB, aac, 6-ib-Cr, vanA, mecA, ermF (of group p2)*
- "Clinical ARGs" of direct health concern, but rarely detected, others can build understanding of how system is operating
- Limitations:
  - Not directly indicative of live organisms, but as **systematically applied, insights into regrowth and risk will be gained**

Methods-Shot gun Metagenomics

- Enabled by next-generation DNA sequencing
- Full profile- No need to choose ARG target *a priori*
- Sequencing depth/cost trade-off
  - Cost is decreasing- likely way of the future
- Still will have to interpret data- sift through and identify ARGs of interest
- Can assemble and identify hosts..



Garner et al., *Scientific Reports*: e.g., Network analysis reveals association of ARGs with horizontal gene transfer elements

Methods- culture *E. coli / Gram positives*

- *E. coli* / Gram positives
- Recommendations in the works- “One Health” approach
- WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance
- EU's Joint Program Initiative on Antimicrobial Resistance (JPIAMR)
- Mark Sobsey member of both
- McLain, Gerrity, others

Case for International Monitoring: e.g. MRSA

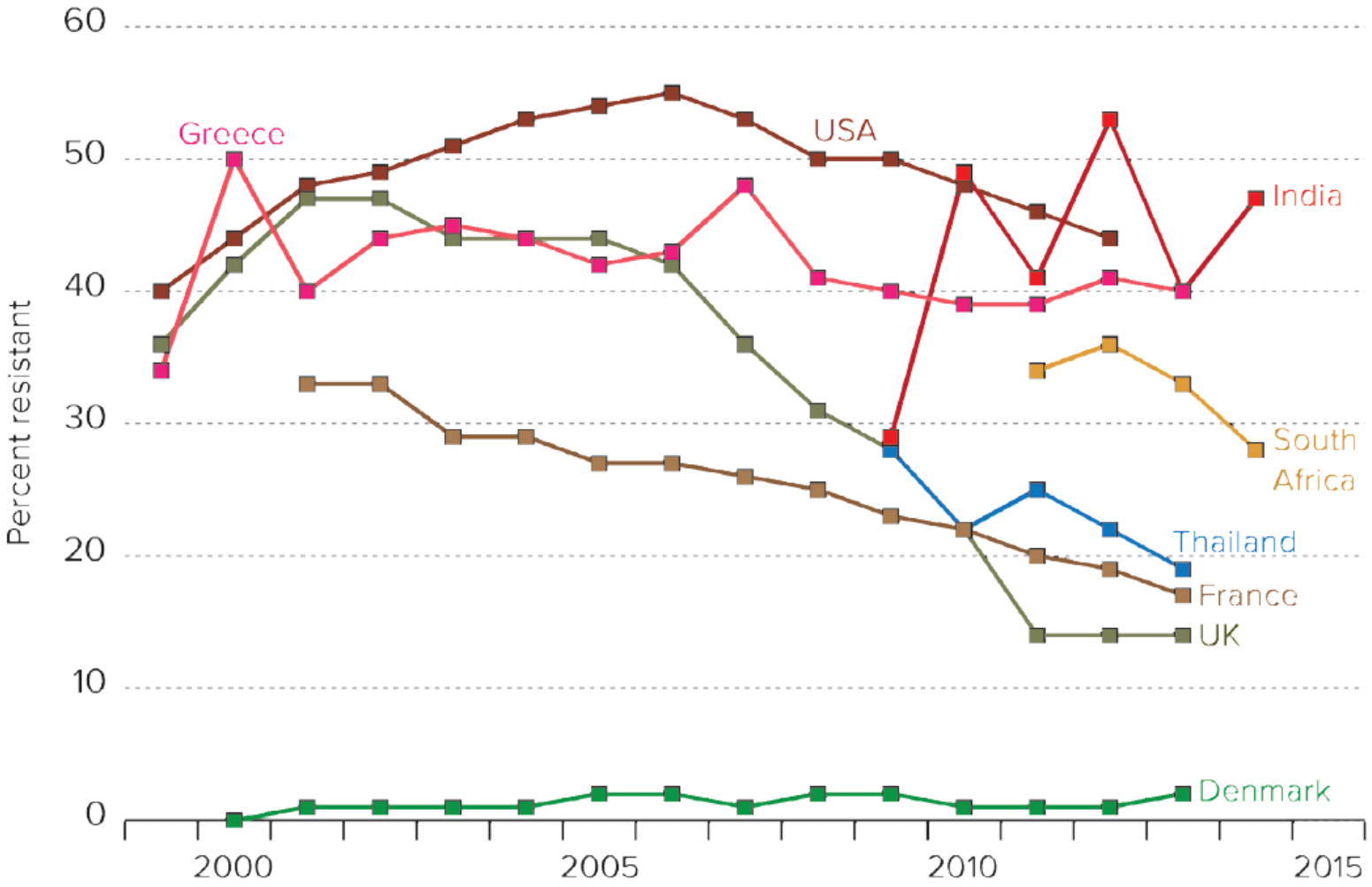
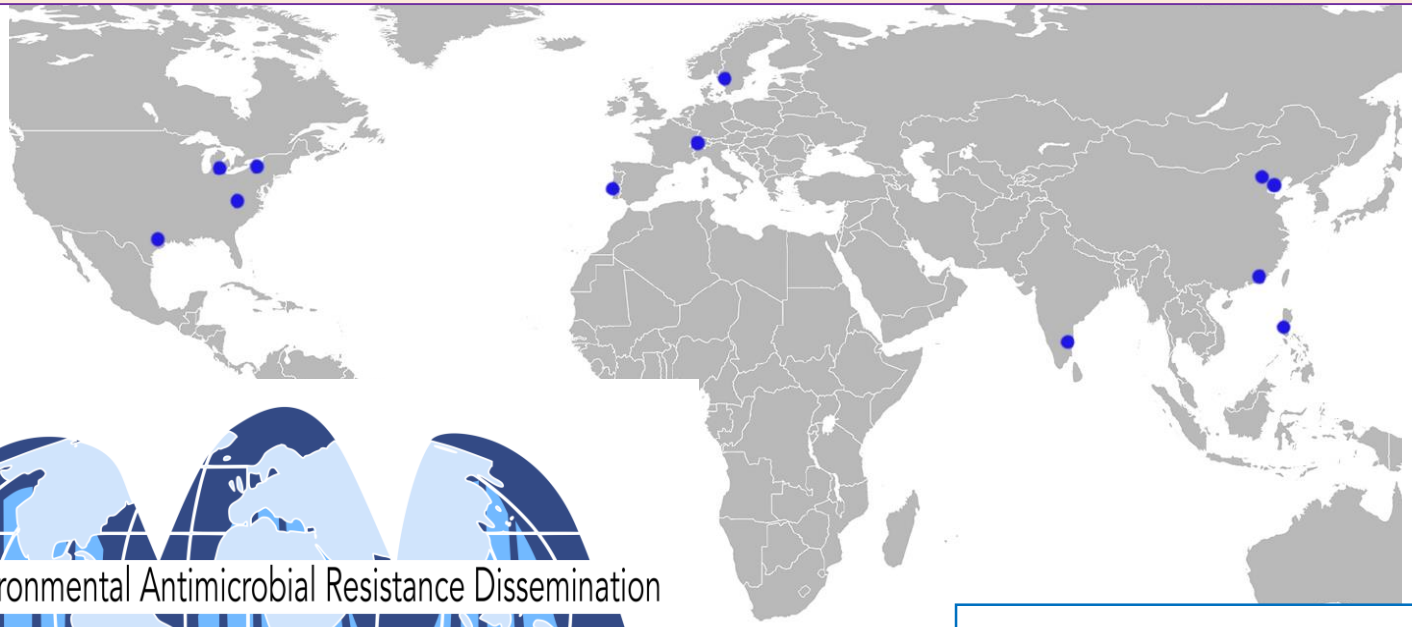


FIGURE ES-1[1]: Percentage of *Staphylococcus aureus* isolates that are methicillin resistant (MRSA) in selected countries, 1999–2014

Source: CDDEP 2015

Global Monitoring: NSF Halting Environmental Antimicrobial Resistance Dissemination (HEARD) PIRE



Halting Environmental Antimicrobial Resistance Dissemination

NSF

Partnership for International Research and Education (PIRE)

Virginia Tech

RICE

MICHIGAN

University at Buffalo
The State University of New York

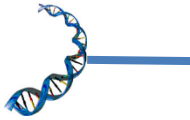
New Risk Frameworks Needed

Adapted from Ashbolt et al. 2013

ARGs

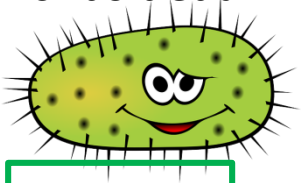
Abs

Metals..

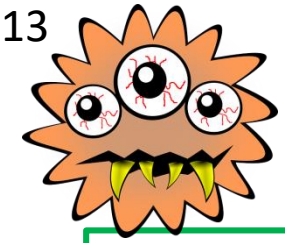


1. Uptake

2. Selection



eARB

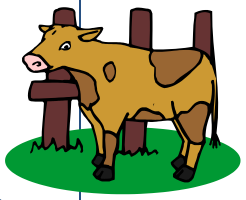


pARB

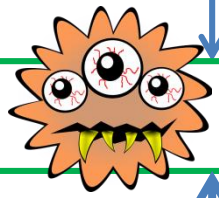
3. uptake, mutation, HGT- humans

4. mutation, HGT- environ.

5. uptake, mutation, HGT- anim.



Development and enrichment of pARB

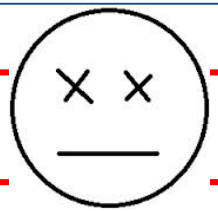


6. Exposure to humans/infection

7. Transfer pARB human/animal

8. Exposure to animal/infection

Antibiotic treatment failure in patients

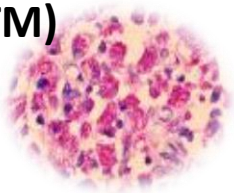


Risk Frameworks: Non-Ingestion Exposures

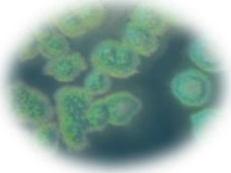
- e.g., Legionnaires' disease and other opportunistic pathogens are the primary source of tap-water outbreak in U.S.
- Similarly, for antibiotic resistance, consider:
  - Inhalation
  - Skin contact
- Colonization- delayed onset, rather than acute illness
  - Current microbial risk frameworks of dose-response may not be appropriate

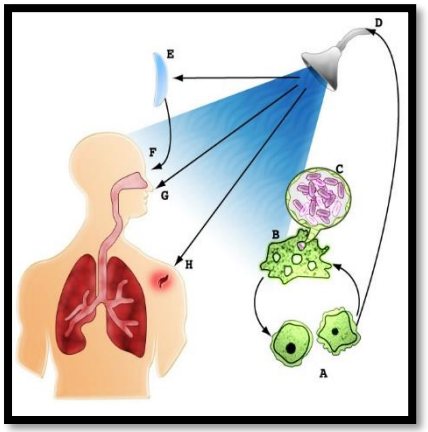
Legionella pneumophila

***Nontuberculous mycobacteria* (NTM)**



Pseudomonas aeruginosa





Mitigation

- Need mitigation options in harmony with other goals:
  - Water reuse and sustainability
  - Removal of other CECs
- Endpoint? **Comparable** to a defined control or **background**
  - ARG diversity, ARG abundance, key clinical ARGs (e.g., CTX-M, KPC, *mcr*-1, NDM-1)
  - Low levels of horizontal gene transfer and multi-drug markers

Review

Management Options for Reducing the Release of Antibiotics and Antibiotic Resistance Genes to the Environment

Amy Pruden,[1] D.G. Joakim Larsson,[2*] Alejandro Amézquita,[3] Peter Collignon,[4,5] Kristian K. Brandt,[6] David W. Graham,[7] James M. Lazorchak,[8] Satoru Suzuki,[9] Peter Silley,[10,11] Jason R. Snape,[12] Edward Topp,[13] Tong Zhang,[14] and Yong-Guan Zhu[15]*

"One Water": Opportunities to Mitigate and Minimize Risks of ARBs and ARGs as CECs

- Management of **Distribution System** and Other Infrastructure

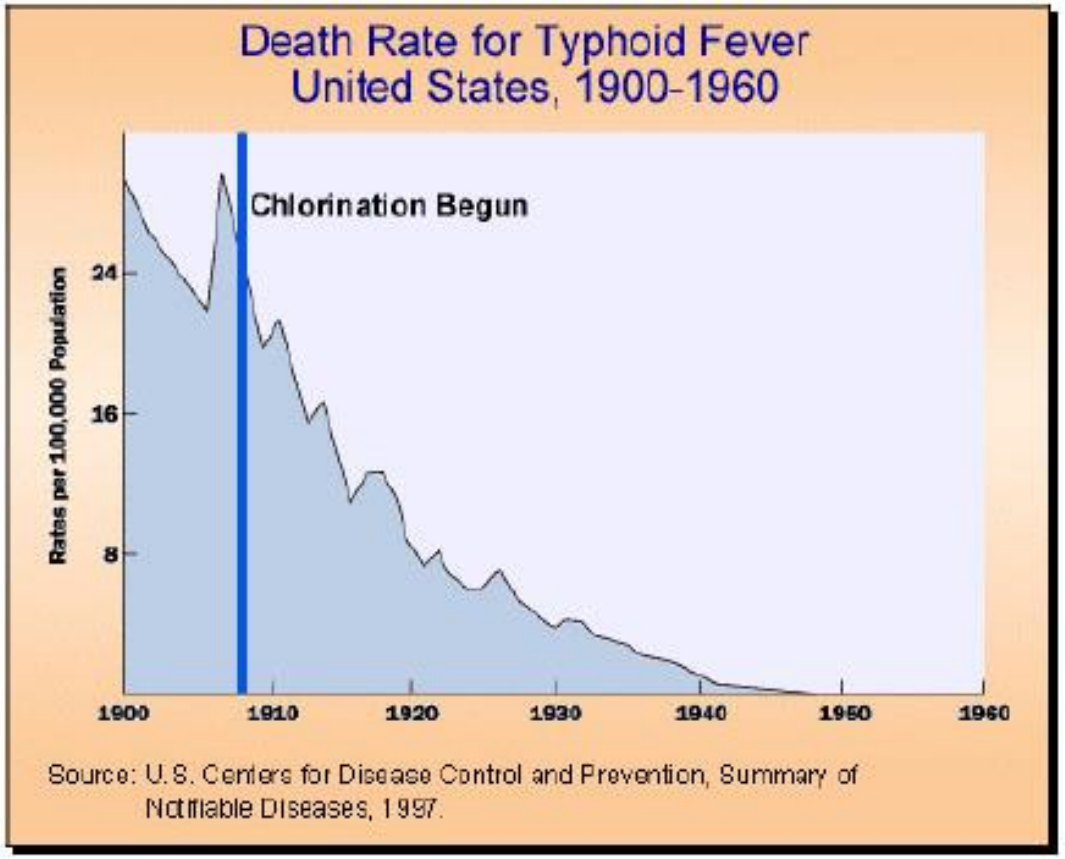
***Minimize Antibiotic + ARGs Inputs** in Wastewater*

*Optimize biological treatment for **minimizing** ARGs and gene transfer*

*UV, disinfectants, Advanced Oxidation Process **damage** of Antibiotics, ARGs*

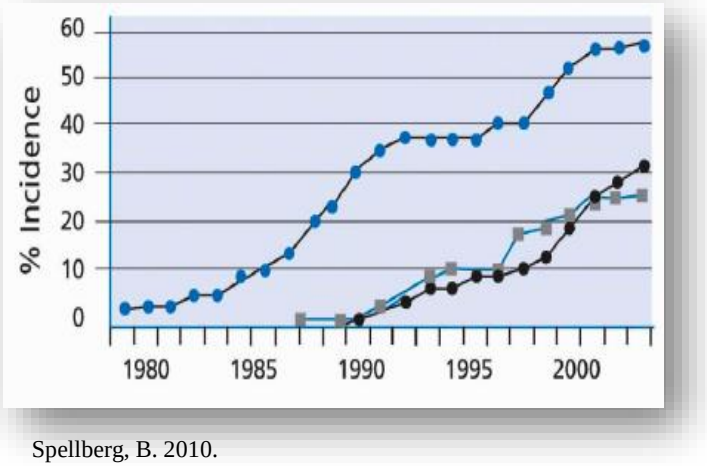
*Membrane **removal** of Antibiotics, ARGs*

Chlorination and Filtration: Water Engineering Eradicated Typhoid Fever



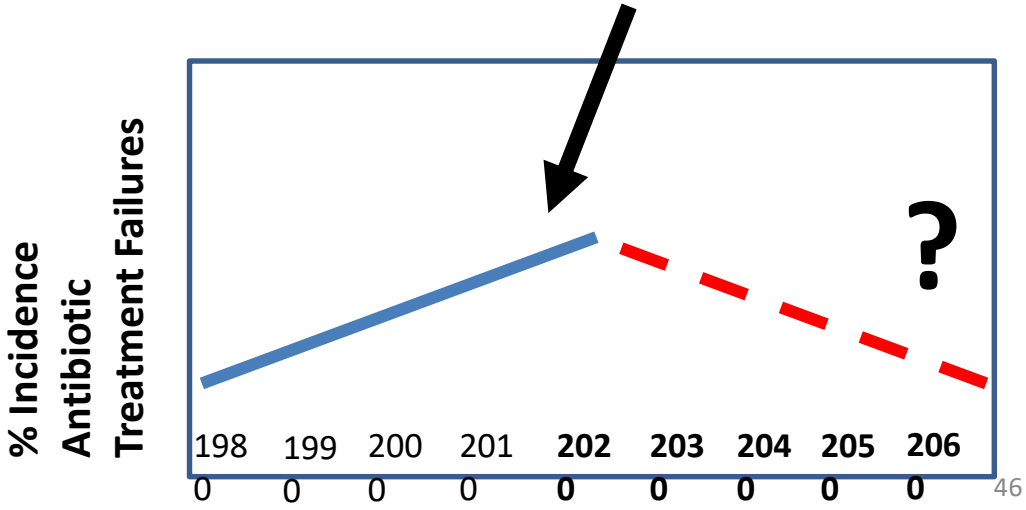
"One Health": Could Water Engineers Once Again Instigate a Public Health Revolution?

Methicillin resistant *Staphylococcus aureus*
Vancomycin resistant *Enterococci*
Fluoroquinolone resistant *Pseudomonas*



Spellberg, B. 2010.

Implementation of Best Management Practices?



Andy Salveson, Carollo

Jeannie McLain, U AZ

Charles Bott, HRSD

Water Reuse

Ni "Joyce" Zhu

Emily Garner

Nicole Fahrenfeld

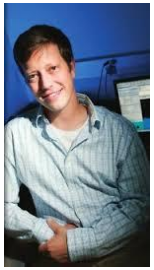
Students

Sudhir Murthy, DC Water

John Novak, VTech

Wastewater

Marc Edwards, VTech



Peter Vikesland, VTech

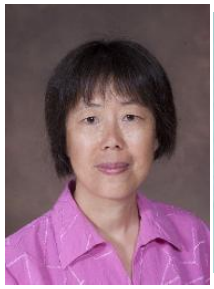
Gustavo Arango, VTech

Liqing Zhang, VTech

Lenny Heath, VTech

Dave Engelthaler, TGen

Bioinformatics



Kang Xia, VTech

Diana Aga, U at Buffalo

Analytical Chemistry

Water Chemistry, Pipes, Sensors

Thank You!!