Antibiotic Resistance Genes

Achieving Our Original Mission of Public Health Protection: Wastewater Treatment in the Era of Emerging Infectious Disease

Amy Pruden, Professor, Virginia Tech

VirginiaTech
Invent the Future
The Charles E. Via, Jr.
Department of Civil and Environmental Engineering

Overview

1. The problem of antibiotic resistance
2. How do bacteria resist antibiotics?
3. What is the evidence for spread of antibiotic resistance through environment via human activities, including water reclamation?
4. What are key knowledge gaps and what can we do?

Media centre

World Antibiotic Awareness Week

16-22 November 2015

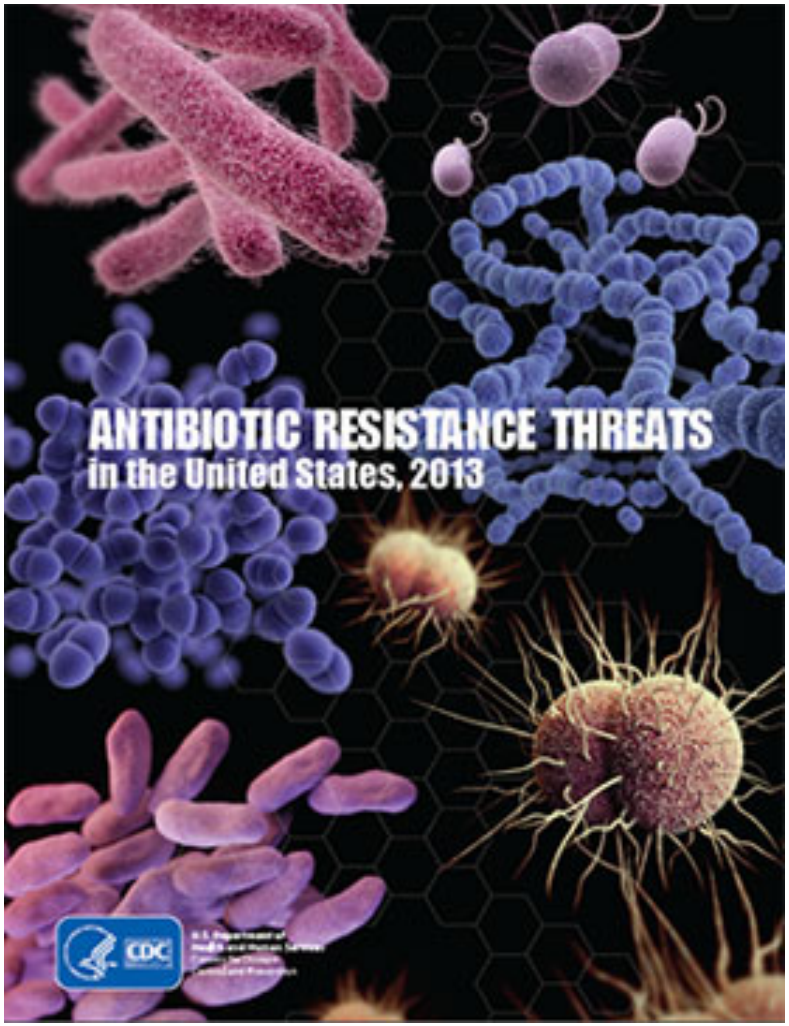
Antibiotics: handle with care

World Antibiotic Awareness Week aims to increase awareness of global antibiotic resistance and to encourage best practices among the general public, health workers and policy makers to avoid the further emergence and spread of antibiotic resistance.

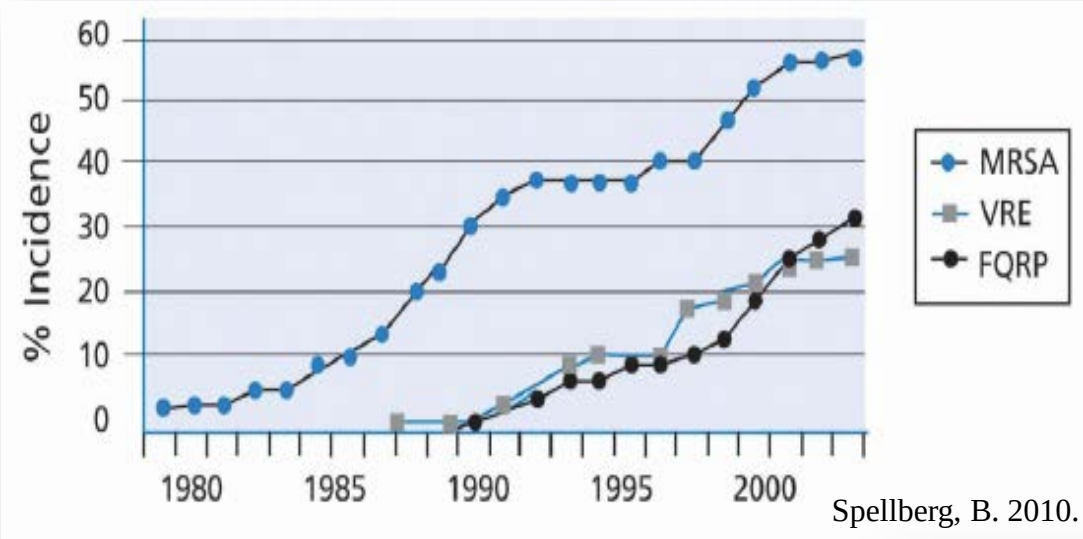
Antibiotic Resistance: The Hard Facts

- 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)
  - Community-acquired MRSA now surpasses hospital-acquired MRSA

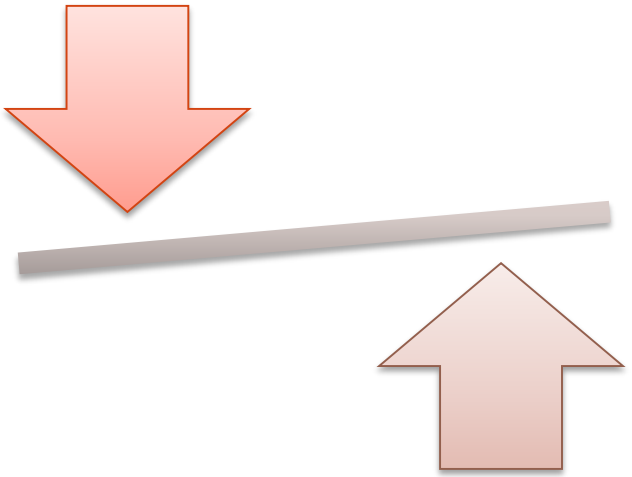
"*Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community*"



Antibiotic Resistance

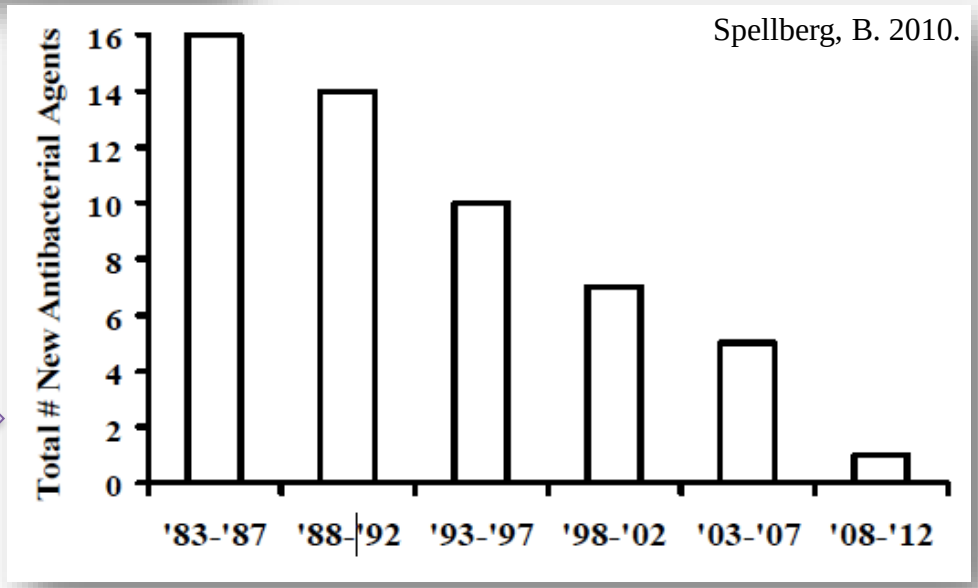


Spellberg, B. 2010.



INCREASED RESISTANCE

DECREASED ANTIBIOTIC DEVELOPMENT



Spellberg, B. 2010.

Need for New Drugs

The New York Times

June 2, 2013

Pressure Grows to Create Drugs for 'Superbugs'

By BARRY MEIER

Government officials, drug companies and medical experts, faced with outbreaks of antibiotic-resistan
raising

SCIENCE | BUSINESS
Innovation intelligence and networking

Bringing together
Research | Industry | Policy

Home News Events Video Reports Communications Partner network Innovation Board About Sign in

Newsletter RSS Contact Us Subscribe Follow @scibus Search

Life Sciences

Horizon 2020: Special Coverage

Related News

Italian biotech accelerator launched with €6M VC investment
18 November 2015

PUBLISHED: 18 NOVEMBER 2015

New reward system needed to encourage pharma to develop new antibiotics

Éanna Kelly, Science|Business

As the first World Antibiotics Awareness week has highlighted, the traditional business model for antibiotics is broken. Incentives are required to promote development of new products, says Luke Moore. What about a lump sum payment?

Luke Moore, Imperial College London

Advertisement

SCIENCE BUSINESS NETWORK

DICAL

Big Data

NATIONAL ACTION PLAN FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

MARCH 2015

Antibiotic Resistance in World News

G8 UK
UNITED KINGDOM 2013

G8 Science Ministers Statement London UK, 12 June 2013

At the meeting we focused on antimicrobial (i.e. anti-viral, anti-bacterial, anti-fungal and anti-parasite) drug resistance as a major health security challenge of the twenty first century. We decided to act concertedly on developing the scientific input necessary to reduce antimicrobial resistance working with existing agencies such as the World Health Organisation and by taking into consideration other activities, to:

v. support international cooperation and sharing of surveillance data to improve global understanding of the spread of antimicrobial drugs resistance;

vi. support theoretical and applied research to better understand the origin, spread, evolution and development of resistance in microorganisms (including viruses, bacteria and parasites) and the role of the innate immune system.

Following MRSA Scare, Eagles vs. Buccaneers Will Not Be Postponed

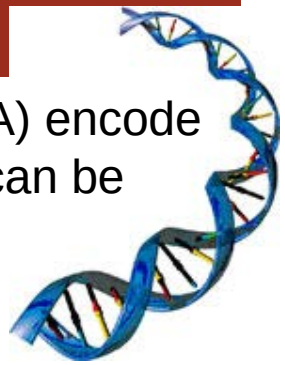
By
Tim Daniels
(Featured Columnist) on October 11, 2013

Al Messerschmidt/Getty Images

After the MRSA (methicillin-resistant Staphylococcus aureus) results came in, the National Football

How do Bacteria Resist Antibiotics?

ARGs (pieces of DNA) encode these functions and can be shared by bacteria



Resistant versus Susceptible *E. coli*

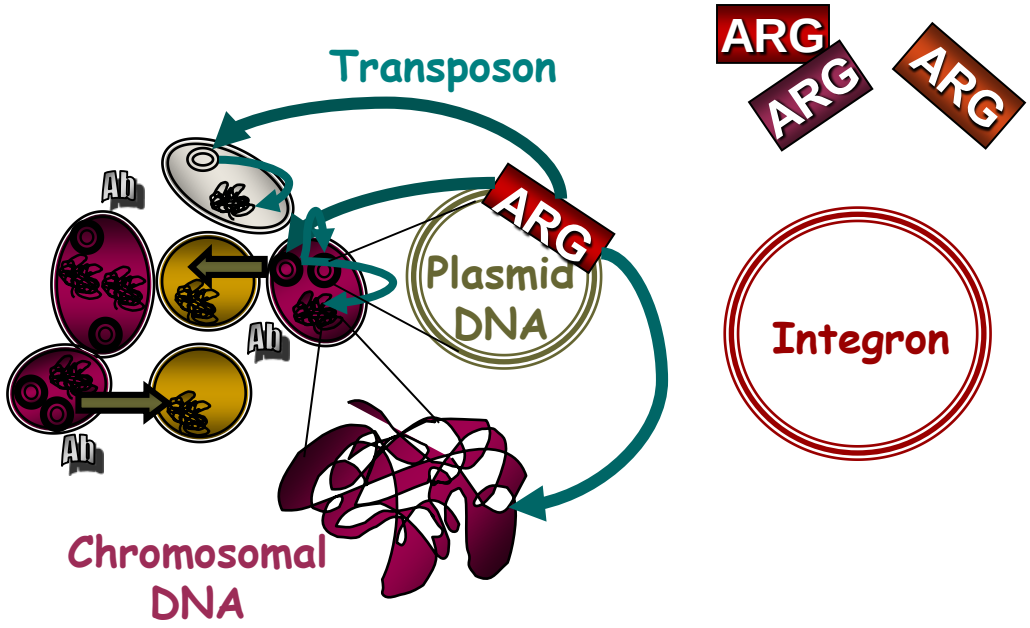
- Kirby Bauer Disc Diffusion Method (*E. coli* SMS 3-5)

Antibiotic Resistance Genes (ARGs)

Antibiotic Resistance

Mutations in chromosome

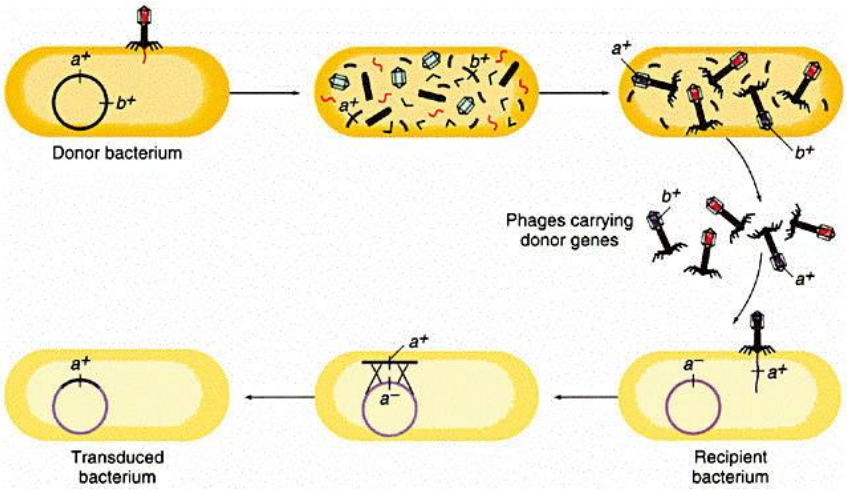
Mobile genetic elements (Antibiotic Resistance Genes or ARG)



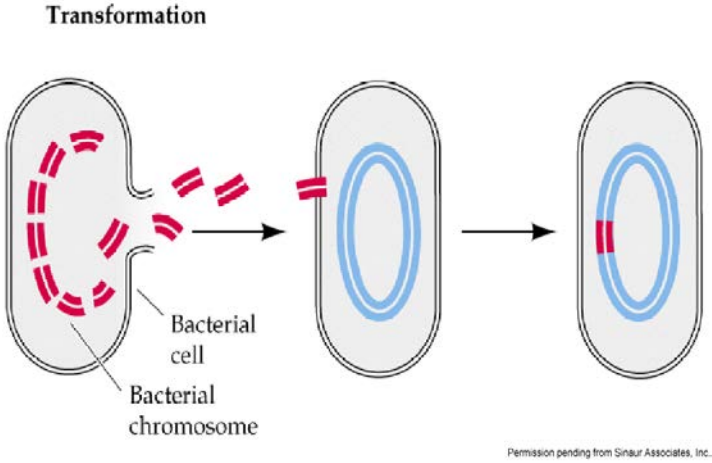
Traditional approach of killing bacteria may not be sufficient- ideally should think about destroying ARGs.

Ways Bacteria Share Genes

Conjugation: Bacterial "mating"

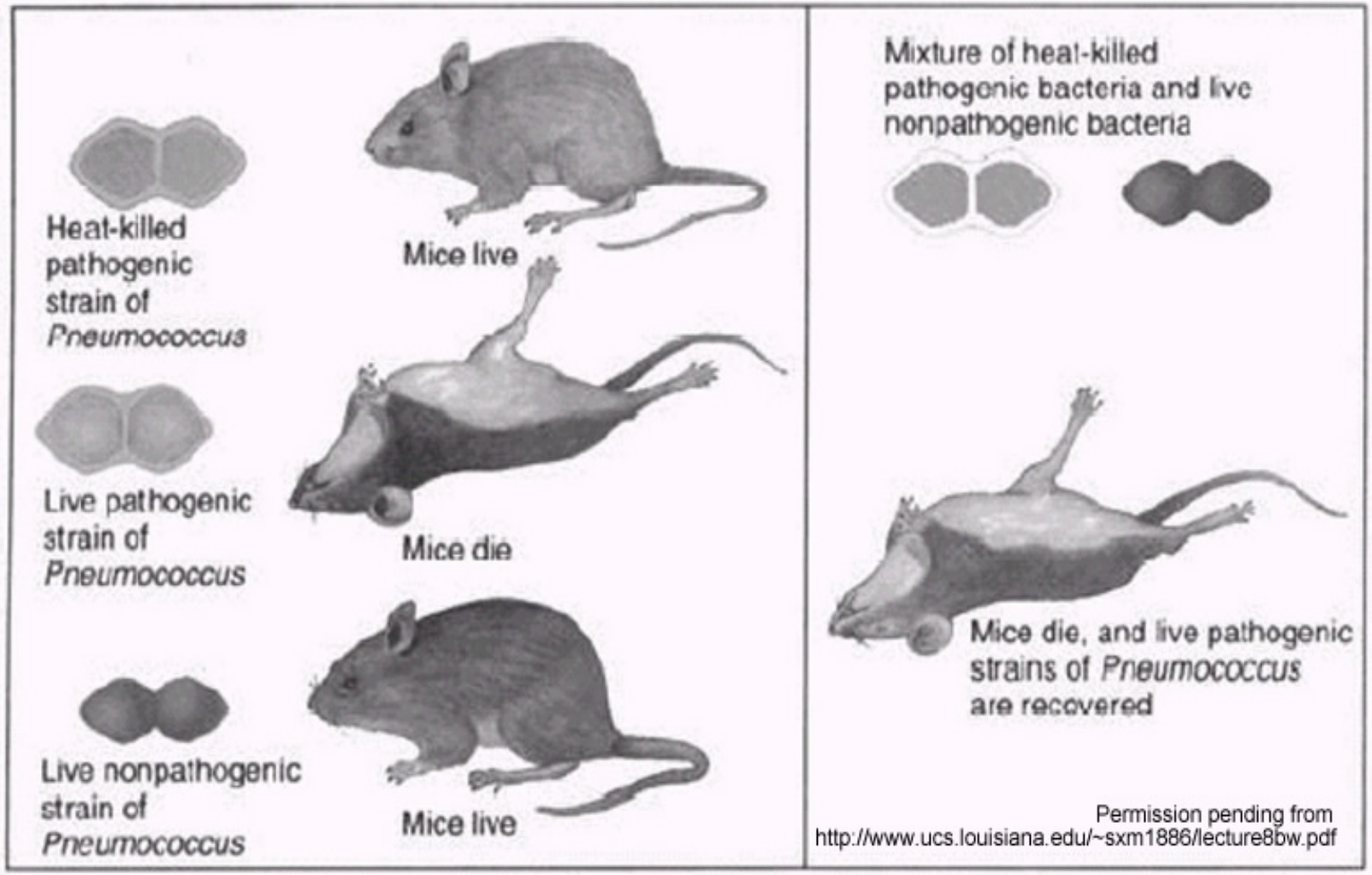


Transduction: Virus Mediated
http://www.youtube.com/watch?v=VX1Ze5edmkE



Transformation: DNA from dead bacteria taken up by live

Griffith's Classic DNA Transformation Experiment (1928)



Permission pending from
http://www.ucs.louisiana.edu/~sxm1886/lecture8bw.pdf

NDM-1 Example

- **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*
- Recently detected in **surface water** in India *(Walsh et al. 2011)*

Klebsiella pneumoniae, Heather Turgeon, *Stroller Derby*

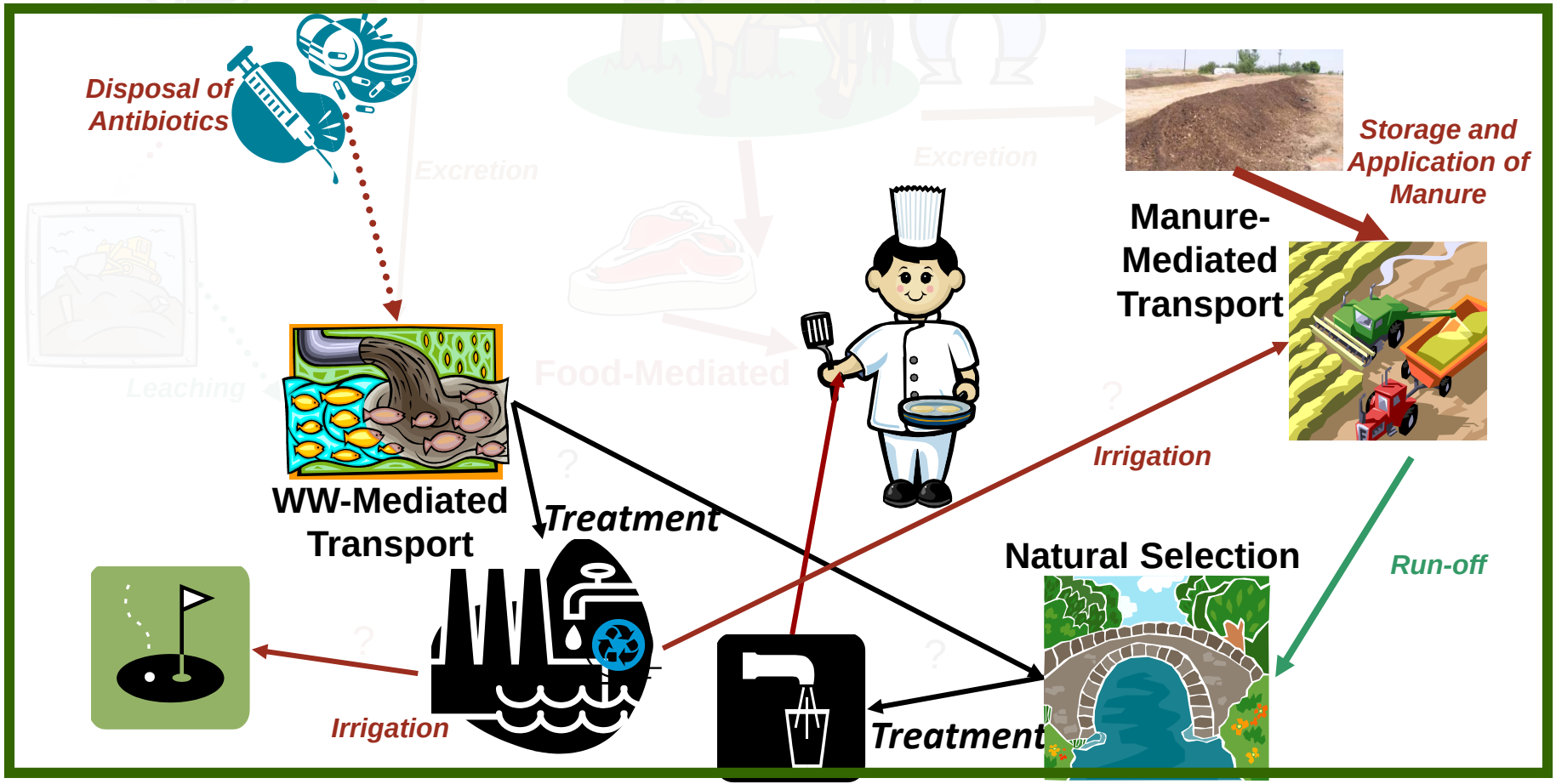
Proliferation of Multidrug-Resistant "Superbugs" (NDM-1 Positive) in Activated Sludge Treatment Plants

Pedro J.J. Alvarez

Clarke Prize Conference, 7 November 2014

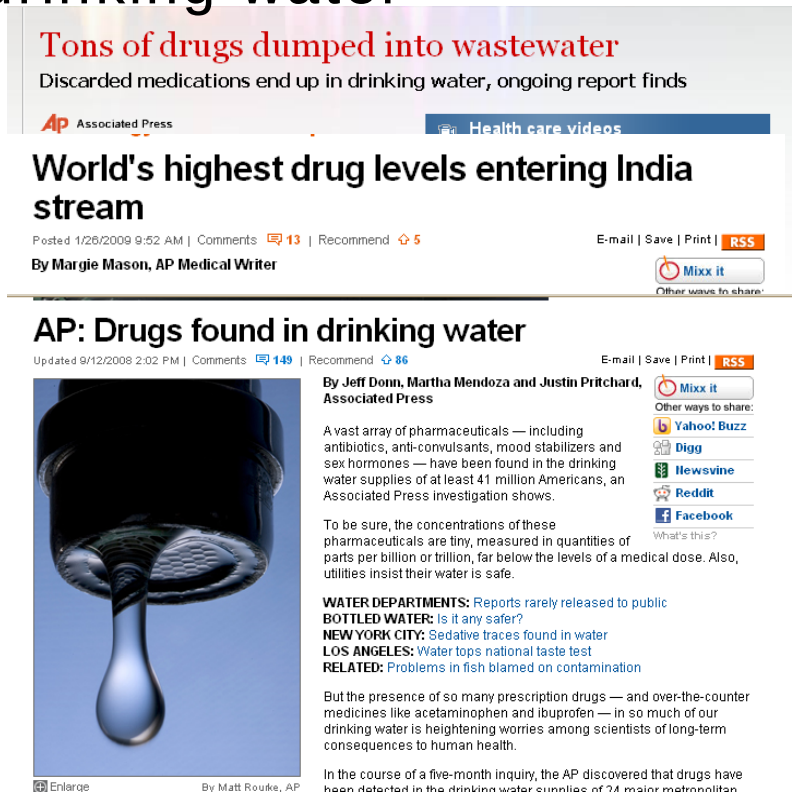
RICE

The Clarke Prize


Disposal of Antibiotics
Storage and Application of Manure
Manure-Mediated Transport
WW-Mediated Transport
Treatment
Irrigation
Natural Selection
Run-off
Irrigation
Treatment

Drugs in our Water

Antibiotics, hormones, and other pharmaceuticals have been found in wastewater, surface water, and even drinking water



Tons of drugs dumped into wastewater

Discarded medications end up in drinking water, ongoing report finds

Associated Press

Health care videos

World's highest drug levels entering India stream

Posted 1/26/2009 9:52 AM | Comments 13 | Recommend 5

E-mail | Save | Print | RSS

By Margie Mason, AP Medical Writer

Mixx it

Other ways to share:

AP: Drugs found in drinking water

Updated 9/12/2008 2:02 PM | Comments 149 | Recommend 86

E-mail | Save | Print | RSS

By Jeff Donn, Martha Mendoza and Justin Pritchard, Associated Press

Mixx it

Other ways to share:

Yahoo! Buzz

Digg

Newsvine

Reddit

Facebook

What's this?

A vast array of pharmaceuticals — including antibiotics, anti-convulsants, mood stabilizers and sex hormones — have been found in the drinking water supplies of at least 41 million Americans, an Associated Press investigation shows.

To be sure, the concentrations of these pharmaceuticals are tiny, measured in quantities of parts per billion or trillion, far below the levels of a medical dose. Also, utilities insist their water is safe.

WATER DEPARTMENTS: Reports rarely released to public
BOTTLED WATER: Is it any safer?
NEW YORK CITY: Sedative traces found in water
LOS ANGELES: Water tops national taste test
RELATED: Problems in fish blamed on contamination

But the presence of so many prescription drugs — and over-the-counter medicines like acetaminophen and ibuprofen — in so much of our drinking water is heightening worries among scientists of long-term consequences to human health.

In the course of a five-month inquiry, the AP discovered that drugs have

Enlarge

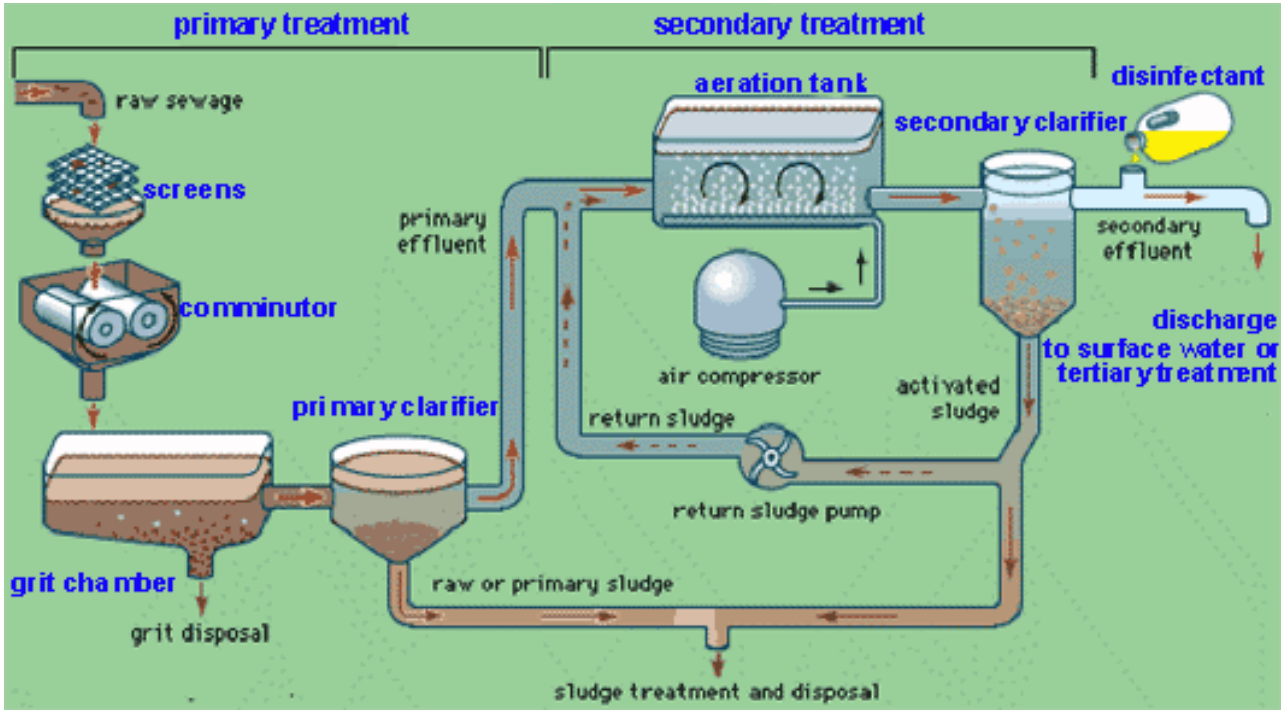
By Matt Rourke, AP

Jason Schneider

Artwork appeared in *Environmental Health Perspectives* (June 2009)

What is Special About WWTPs?

- Receive antibiotics and pathogens
- Concentrated, highly active bacteria
- High potential for sharing of genes



Two Main Outputs:

1. Reclaimed Water
2. Biosolids

www.earthplace.com

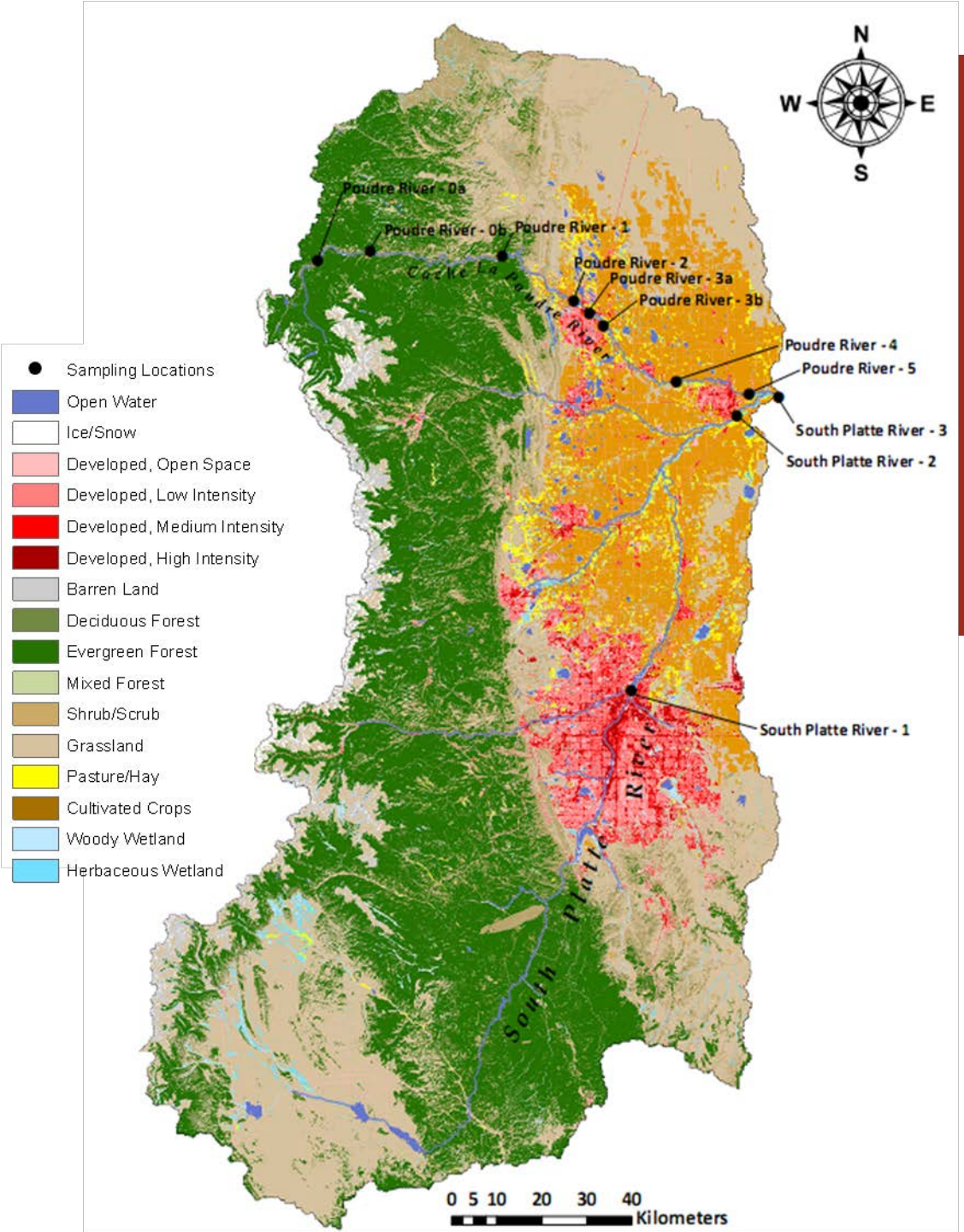
Evidence for Environmental Sources of Antibiotic Resistance and Effects of Human Activities

?

Assaying Human Impact on ARGs in the Poudre River

- Primary source is snowmelt
- No major tributaries

- Well-zonated
- Well-characterized in terms of antibiotics



Land Use in the South Platte River Basin

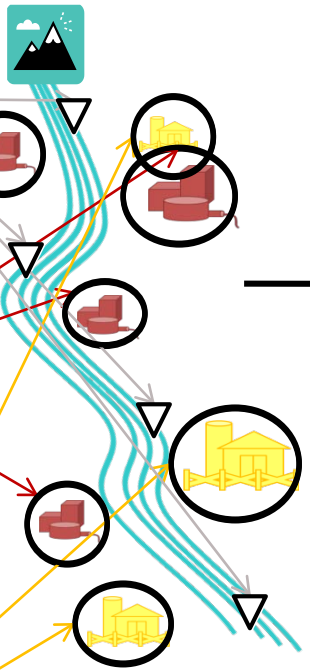
Storteboom et al.
ES&T 2010

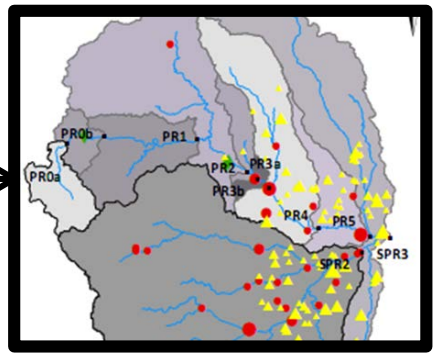
Seeking a Quantitative Relationship: Geospatial Analysis

Handheld GPS unit

EPA Envirofacts Warehouse

Environmental Risk Assessment and Management System (eRAMS)





Geographical Information Systems (GIS) Database

Delineate the subwatershed of each sampling location

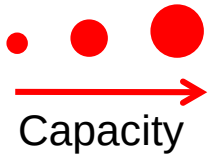
Calculate distances from sources to river sampling sites

Poudre and South Platte

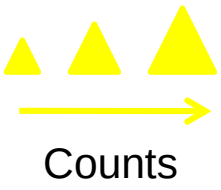
PR0a	Elephant Rock, Roosevelt National Forest
PR0b	Profile Rock, Roosevelt National Forest
PR1	Greyrock Trailhead, Roosevelt National Forest
PR2	Shields St. Bridge, Fort Collins
PR3a	Mulberry St. Bridge, Fort Collins
PR3b	Drake Reclamation Facility, Fort Collins
PR4	95th Avenue Bridge, Weld County
PR5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

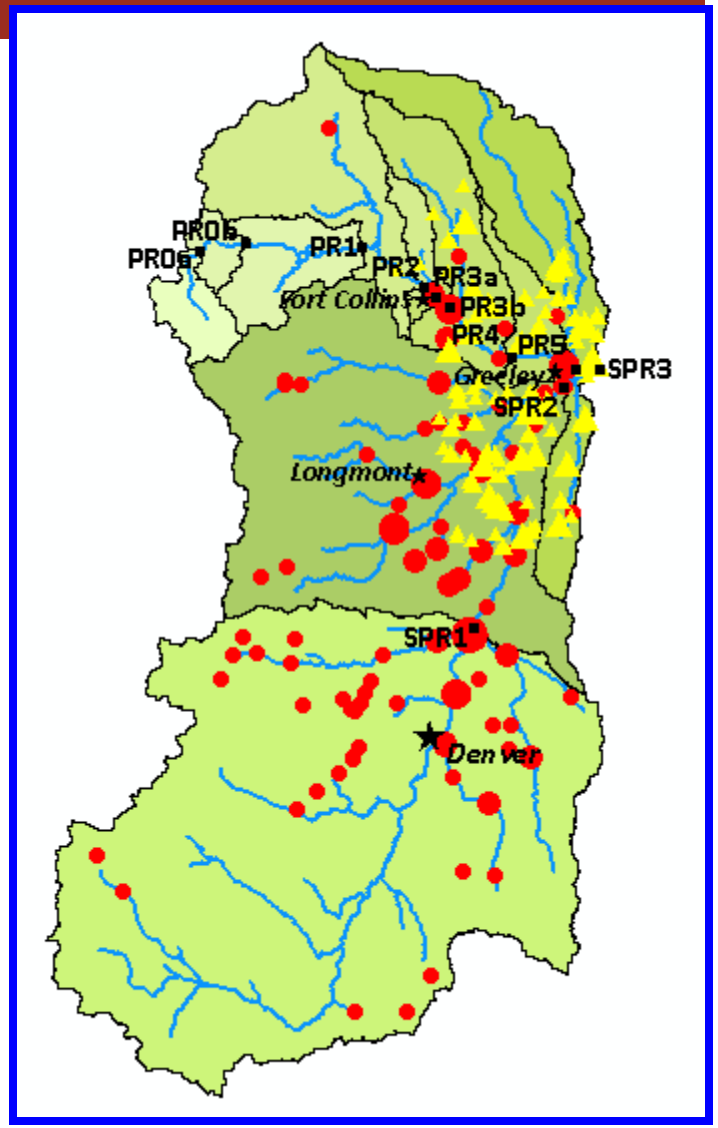
Legend

Wastewater Treatment Plants



Animal Feeding Operations





Constructing Spatial Variables

Inverse Distance Weighted (IDW) Count

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

General Linear Regression Models

1. $\log(y) = a_0 + a_1 \sum_{i=1}^{m} X_{AFO,i}$ (AFOs only)

2. $\log(y) = a_0 + a_1 \dfrac{\sum_{i=1}^{m} \frac{X_{AFO,i}}{D_{AFO,i}}}{\sum_{i=1}^{m} \frac{1}{D_{AFO,i}}}$ (weighted AFOs)

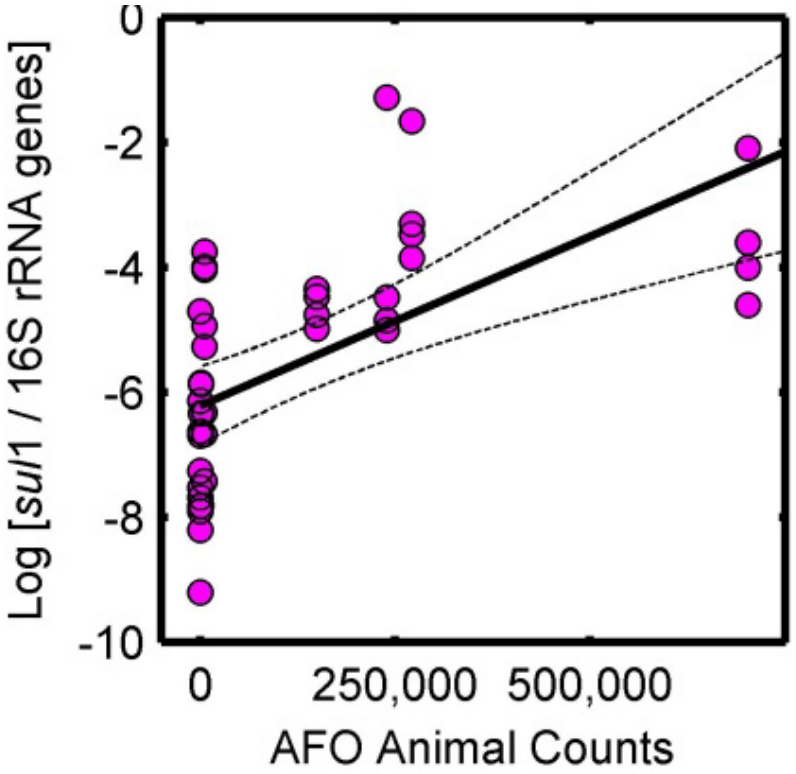
3. $\log(y) = a_0 + a_1 \sum_{i=1}^{n} X_{WWTP,i}$ (WWTPs only)

4. $\log(y) = a_0 + a_1 \dfrac{\sum_{i=1}^{m} \frac{X_{AFO,i}}{D_{AFO,i}}}{\sum_{i=1}^{m} \frac{1}{D_{AFO,i}}} + a_2 \dfrac{\sum_{i=1}^{n} \frac{X_{WWTP,i}}{D_{WWTP,i}}}{\sum_{i=1}^{n} \frac{1}{D_{WWTP,i}}}$

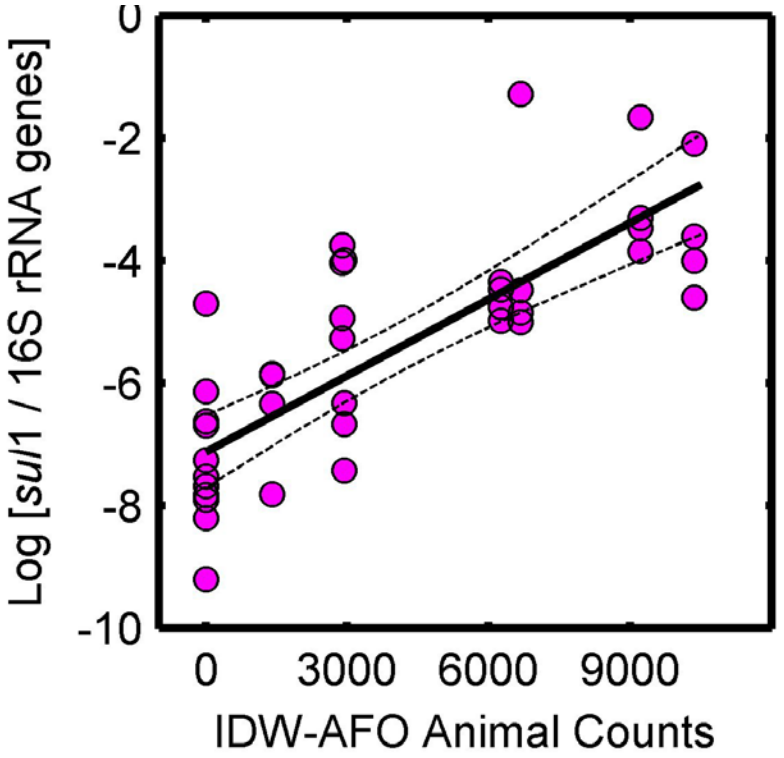
(weighted AFOs + weighted WWTPs)

y=ARGs/16S

Correlation of *sul*(I) with AFOs



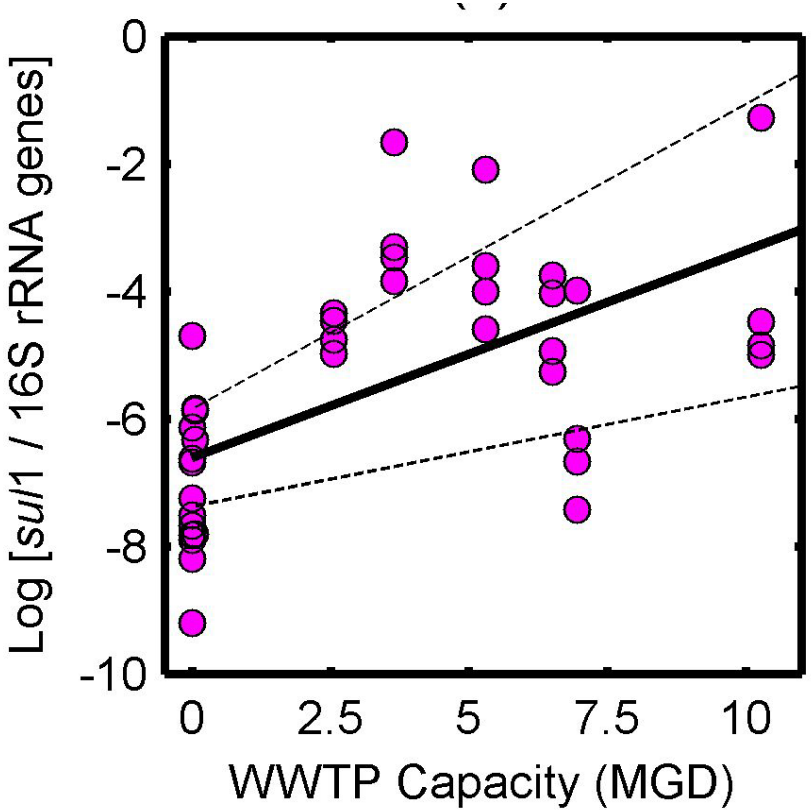
(R^2=0.35, p<0.001)



(R^2=0.64, p<0.001)

Pruden et al. *ES&T* 2012

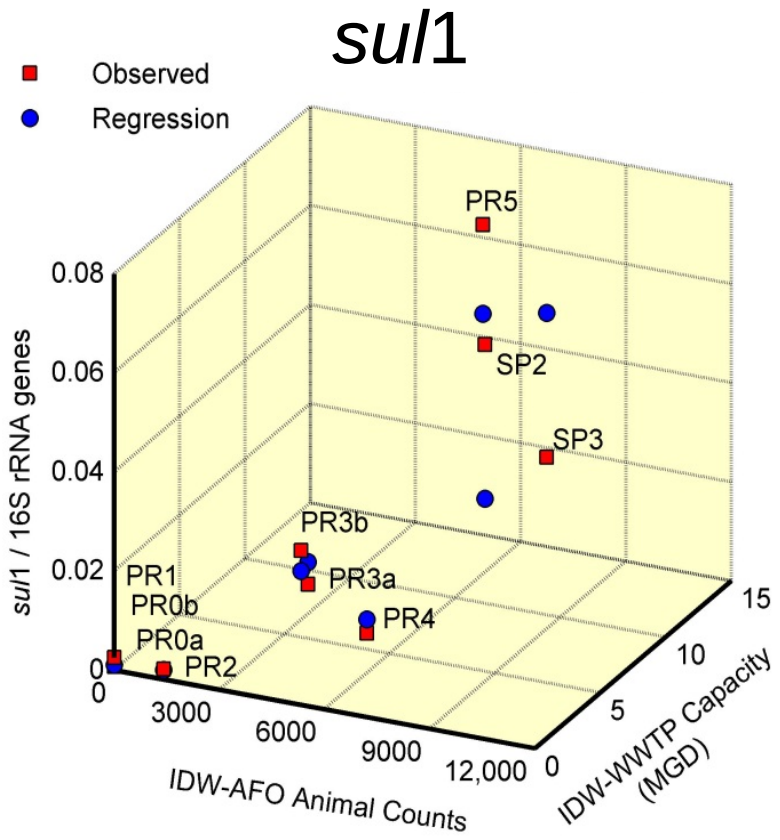
Correlation of *sul*(I) with WWTPs

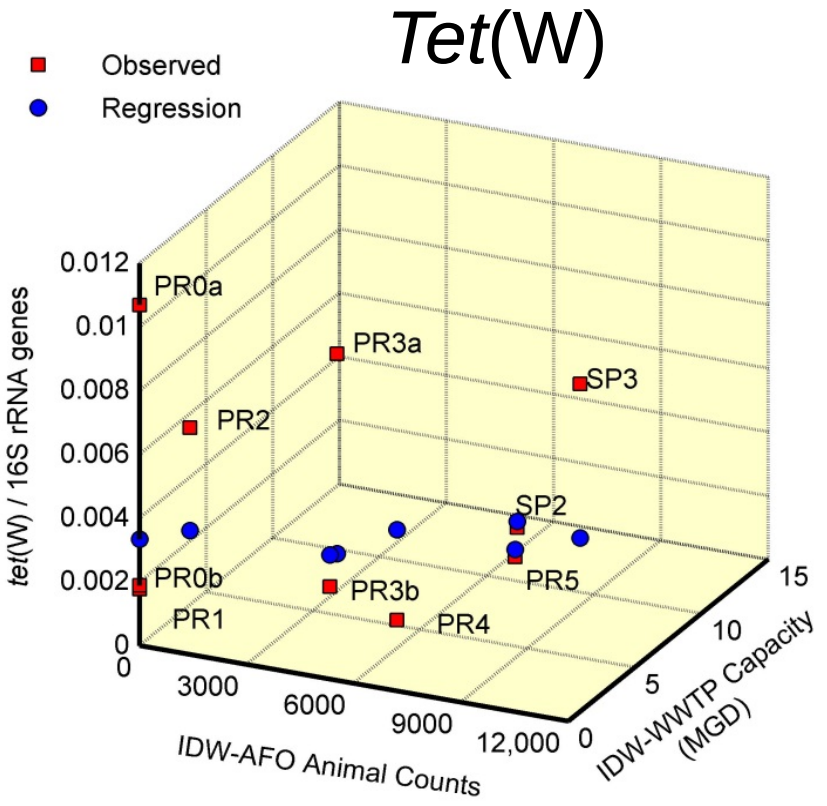


(R^2=0.34, p<0.001)

Pruden et al. *ES&T* 2012

Multivariate Correlation with AFOs and WWTPs





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

(R^2=0.23, p=0.39)

Human Impact on Resistome: More Evidence

- Study went… viral…phenomenon highly replicated
  - ARGs elevated in Duluth harbor receiving WWTP effluent (LaPara et al. *ES&T* 2011)
  - Soil ARGs elevated by biosolids amendment (Munir and Xagoraraki 2011)
  - Viable MRSA and vancomycin-resistant *Enterococci* have been detected in reclaimed water intended for reuse (Sapkota and colleagues 2012, 2013)

Resistance Hot Spot in India

Published online 16 February 2011 | Nature | doi:10.1038/news.2011.46

News

Dumped drugs lead to resistant microbes

A continual discharge of antibiotic-contaminated water has created a hotspot of bacterial antibiotic resistance in an Indian river.

Naomi Lubick

High levels of antibiotic resistance have been found in bacteria that live downstream from a waste-water treatment plant in Patancheru, near Hyderabad in India[1].

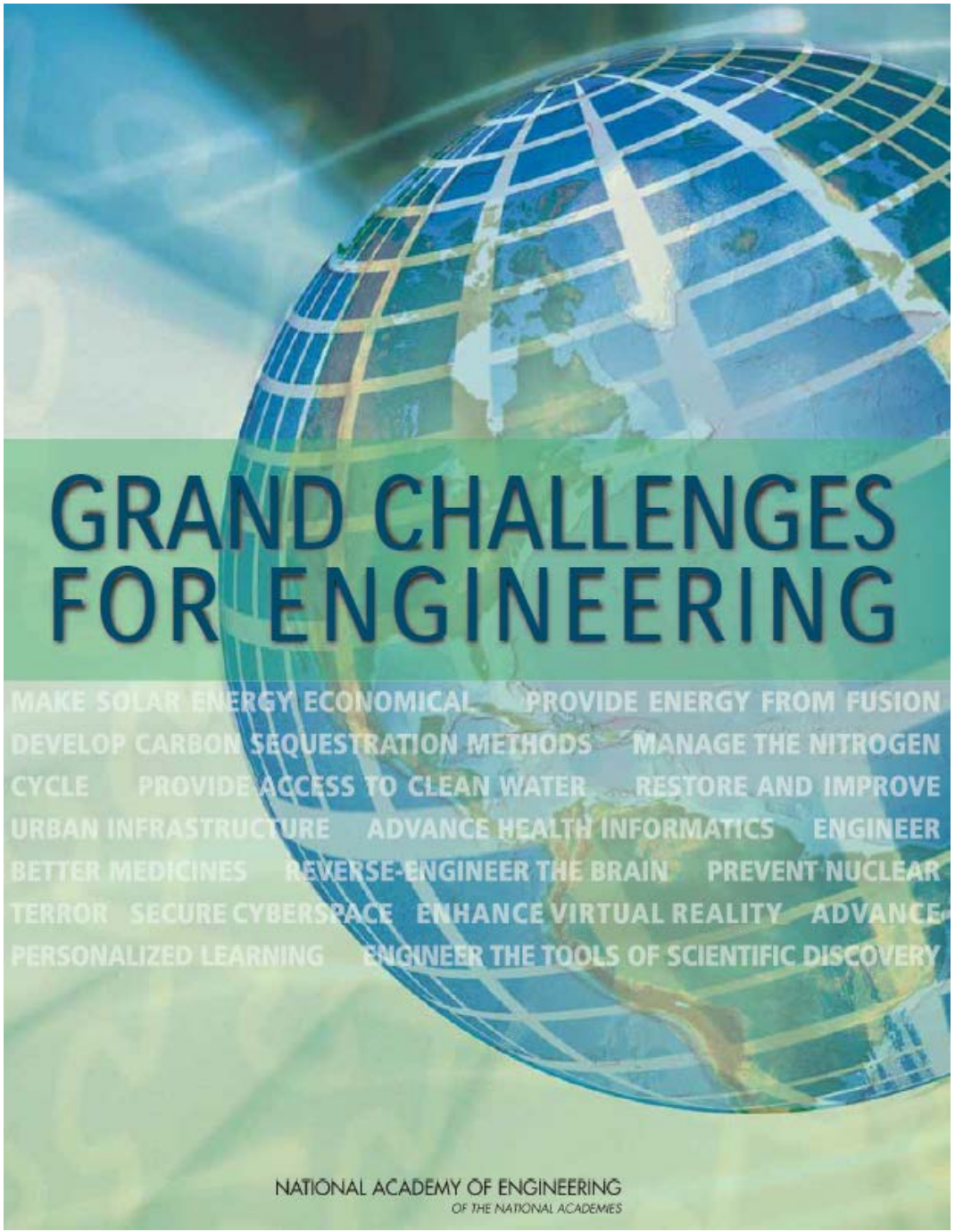
Two years ago, Joakim Larsson of the University of Gothenburg, Sweden, and his colleagues reported that the treatment plant released drugs in its effluent water at levels sometimes equivalent to the high doses that are given therapeutically[2]. The antibiotic-containing water reaching the plant came from 90 bulk pharmaceutical manufacturers in the region, near Hyderabad, they determined. The researchers wondered what might be happening to bacteria in the environment exposed to these drugs.

Antibiotics discharged from a waste-water plant in India have created a hotspot of bacterial resistance. *AP Photo/Mahesh Kumar A*

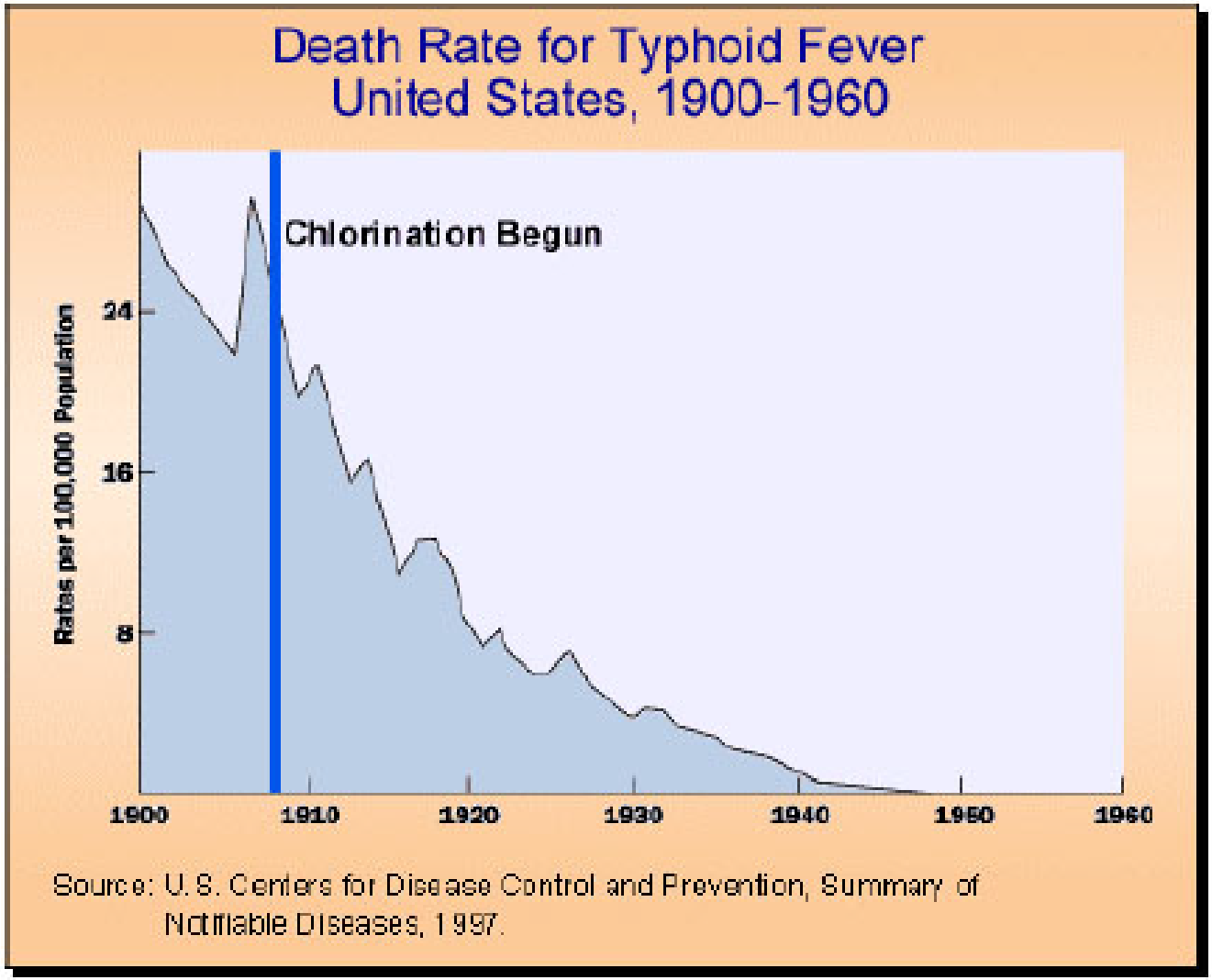
What Can we Do?

Provide access to clean water

Restore and improve urban infrastructure

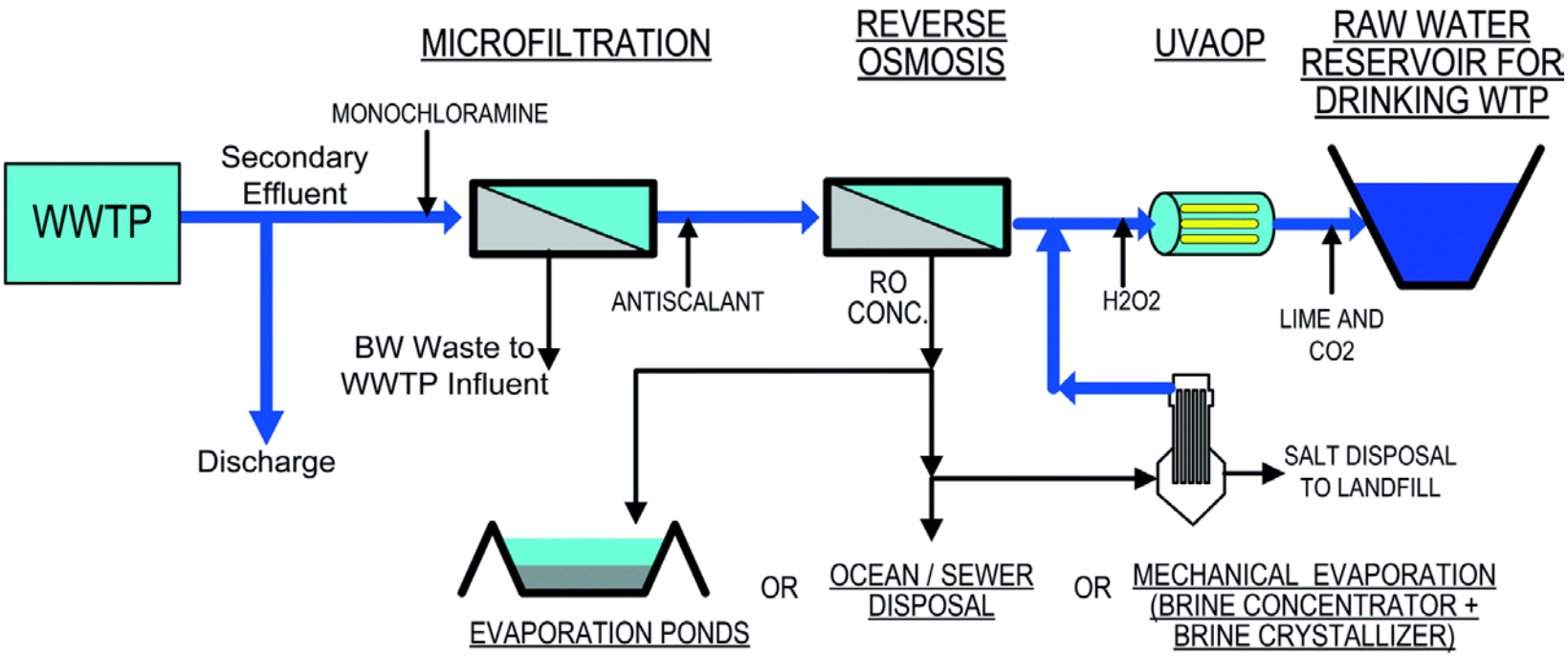


Our Heritage of Protecting Public Health



How Can We Enhance Water Reclamation to Treat ARGs?

e.g., Direct Potable Reuse



Schimmoller et al. *ES:WR&T* 2015
CH2MHill

Optimizing ARG Disinfection

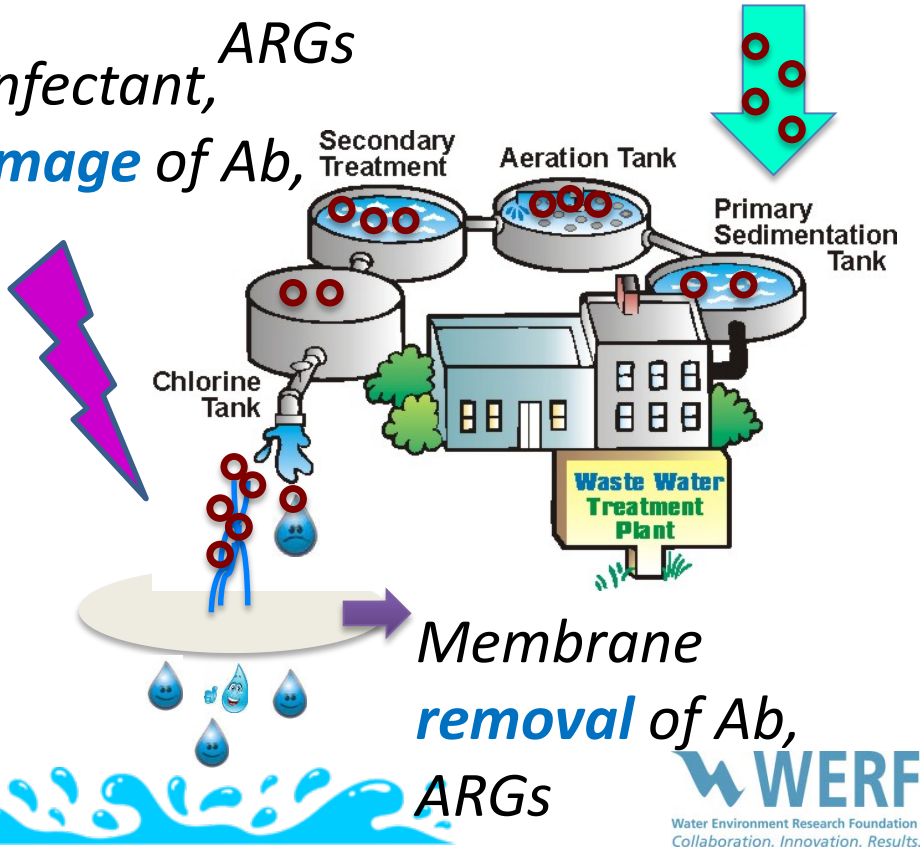
- Can disinfection be optimized to destroy ARGs? (Dodd, 2012)
  - Chlorination
  - UV treatment
  - Advanced Oxidation Processes (AOPs)
- Disinfection/AOPs can also be effective for removal of antibiotics

Management of Antibiotic Resistance Risk in Sustainable Water Systems

- DNA/Ab **Removal**
- DNA/Ab **Damage**
- Management of **Distribution System** and Other Infrastructure

Wastewater containing antibiotics (Ab) + ARGs

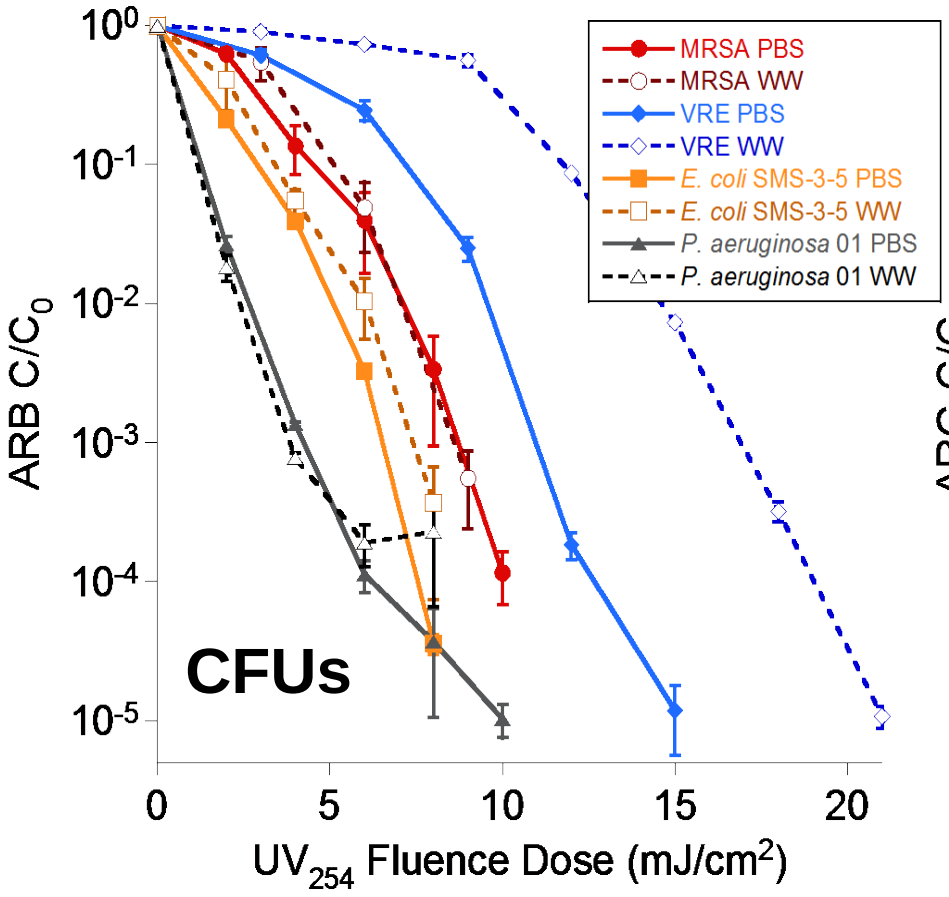
*UV, disinfectant, AOP **damage** of Ab, ARGs*

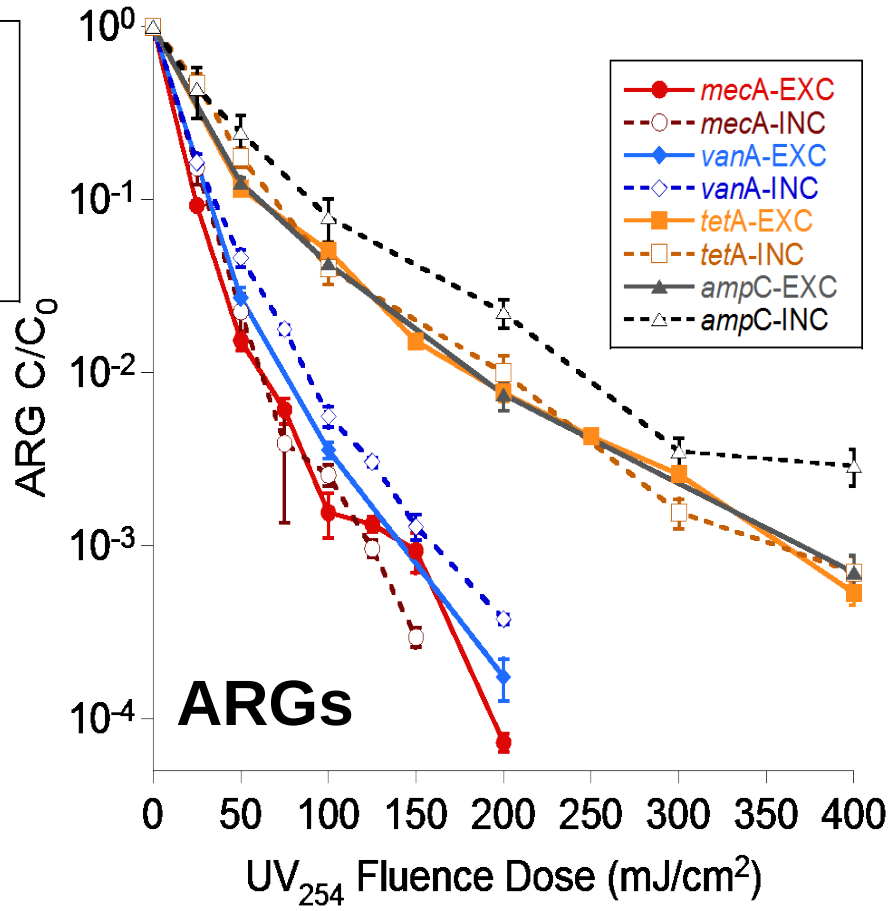


*Membrane **removal** of Ab, ARGs*

WERF
Water Environment Research Foundation
Collaboration. Innovation. Results.

UV Treatment of ARGs

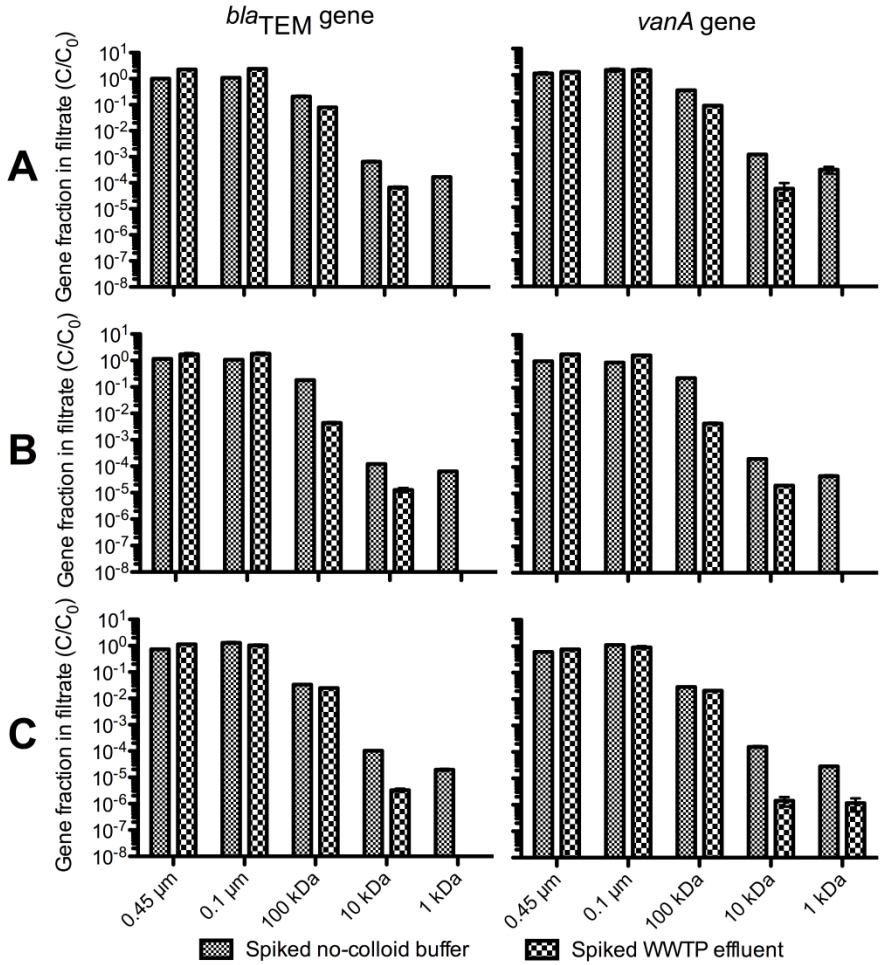




McKinney & Pruden ES&T *2012*

Membrane Treatment of ARGs

- ARG removal by ultrafiltration better than expected based on size
- ARG removal enhanced in wastewater effluent matrix

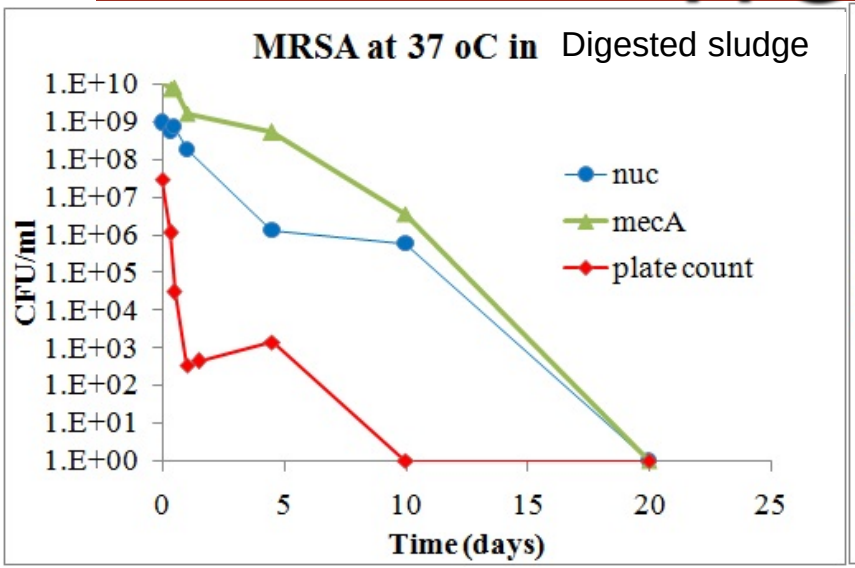


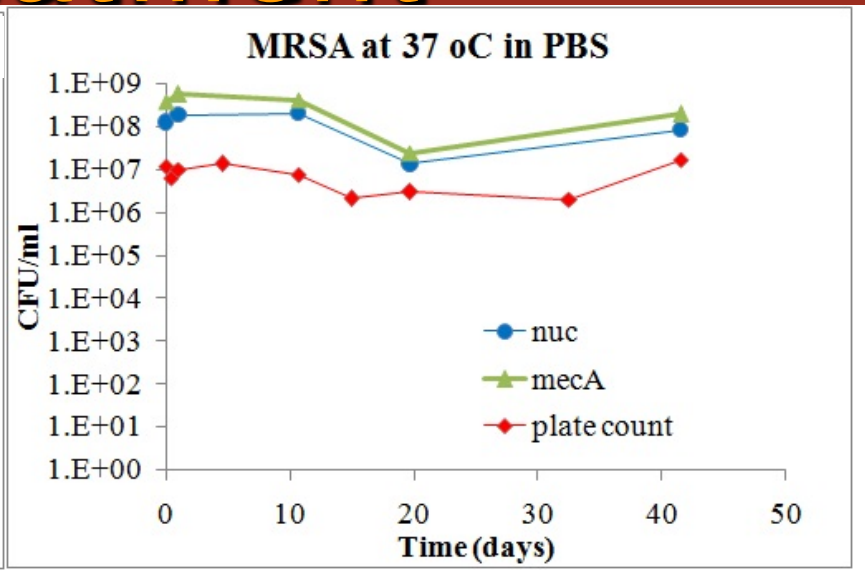
WERF Project Report INFR8SG09; Riquelme Breazeal et al. *Water Res* 2013

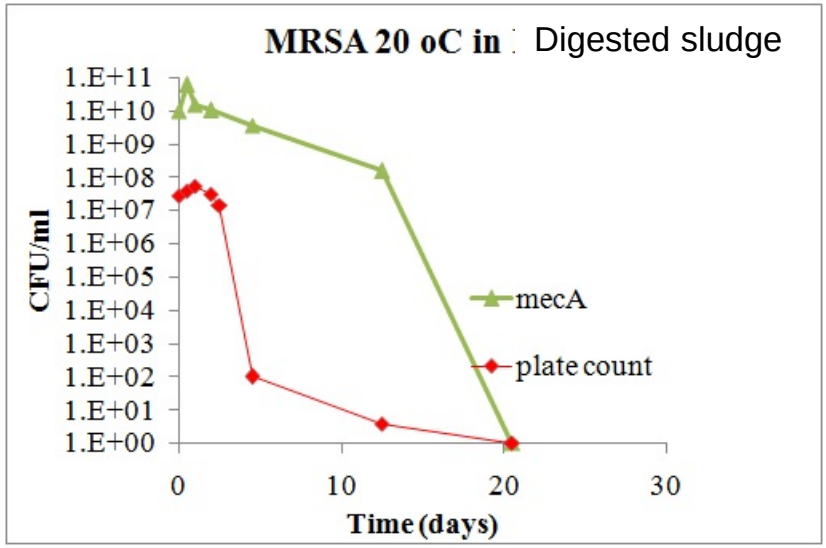
Biological Treatment

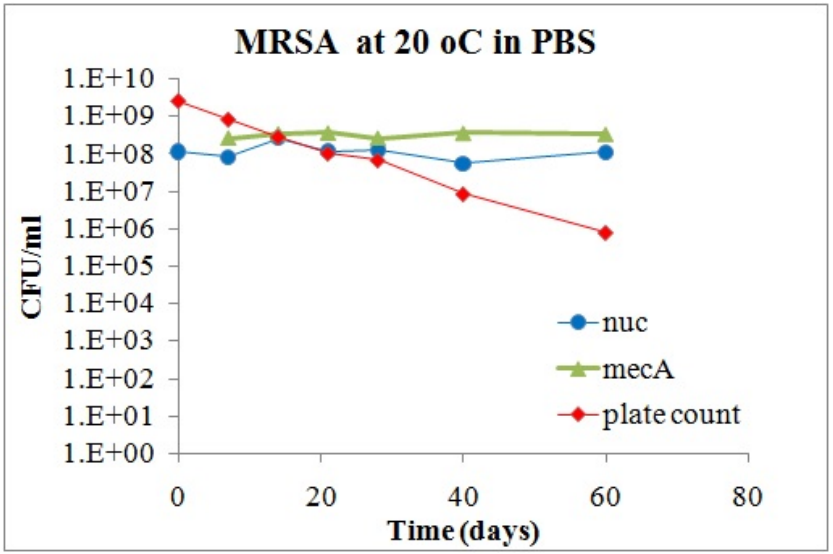
- Examining treatment conditions
  - varying Solids Retention Time- Pending WERF project (Gerrity et al. UNLV and U AZ)
  - Dominic Frigon (McGill University)
- Improving Anaerobic Digestion
  - Tim LaPara
  - Ma, Wilson, Novak, Riffat, Aynur, Murthy, Pruden (2011) "*Effect of various sludge digestion conditions on sulfonamide, macrolide, and tetracycline resistance genes and class I integrons*"

WERF U1R12: Biosolids Treatment









Paul L. Busch Award Research

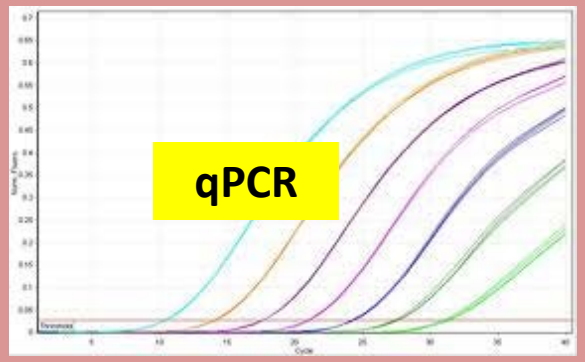
- Compare ARGs in reclaimed and potable water distribution systems
  - Potable water is an important “control”
  - Potable water distribution system management can inform distribution of recycled water
  - Examine role of microbial re-growth
  - Use next generation DNA sequencing for deep insight into microbial community and ARGs
  - Compare with culture-based methods

Distribution System Survey

Sample Collection

Microbiology Methods

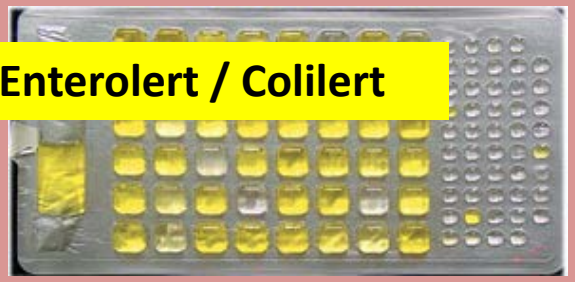
OPs

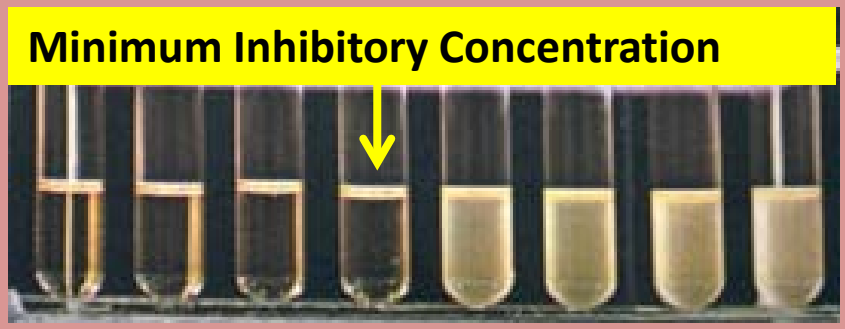


ARGs

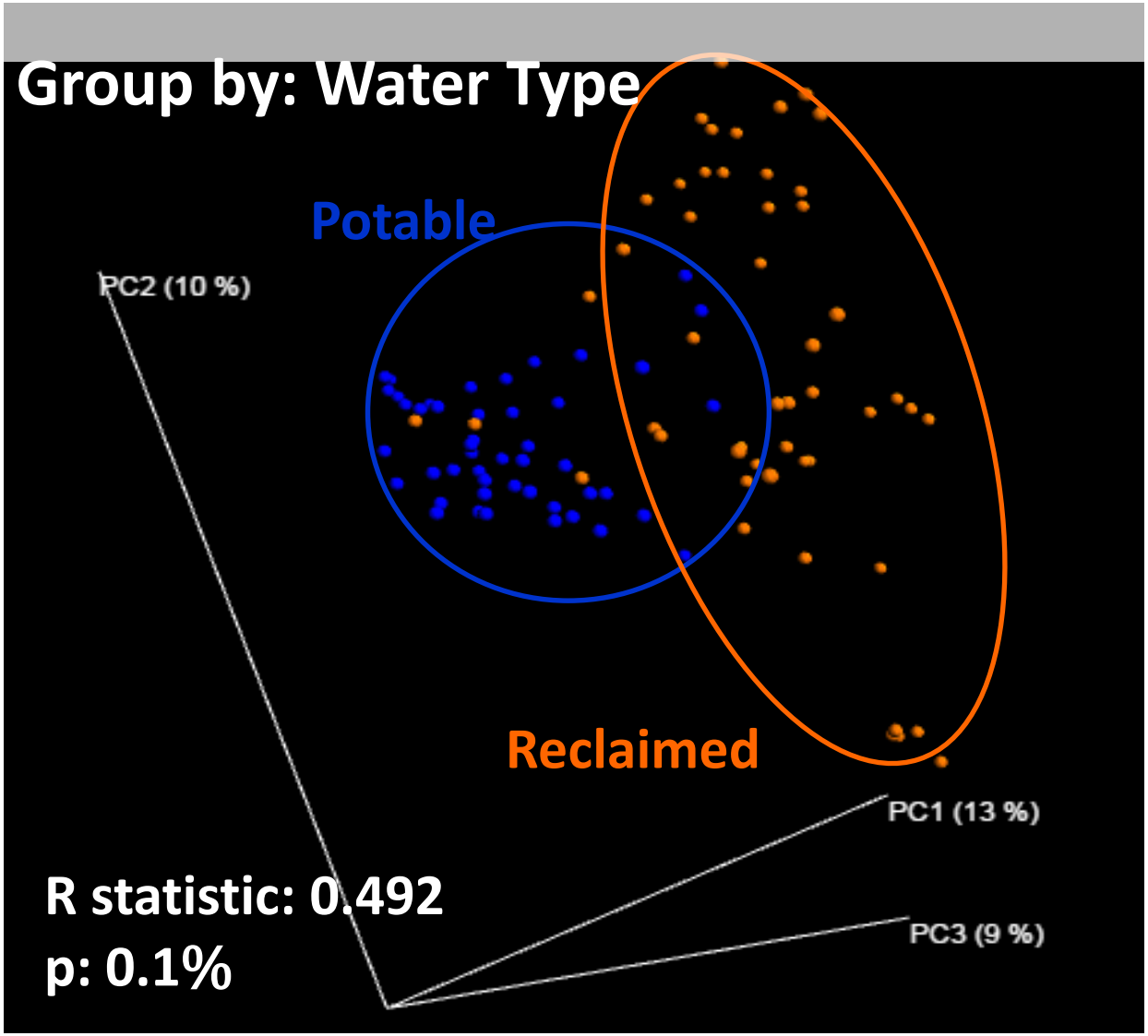
MG-RAST
metagenomics analysis server

E. coli and *Enterococcus* resistance profiles

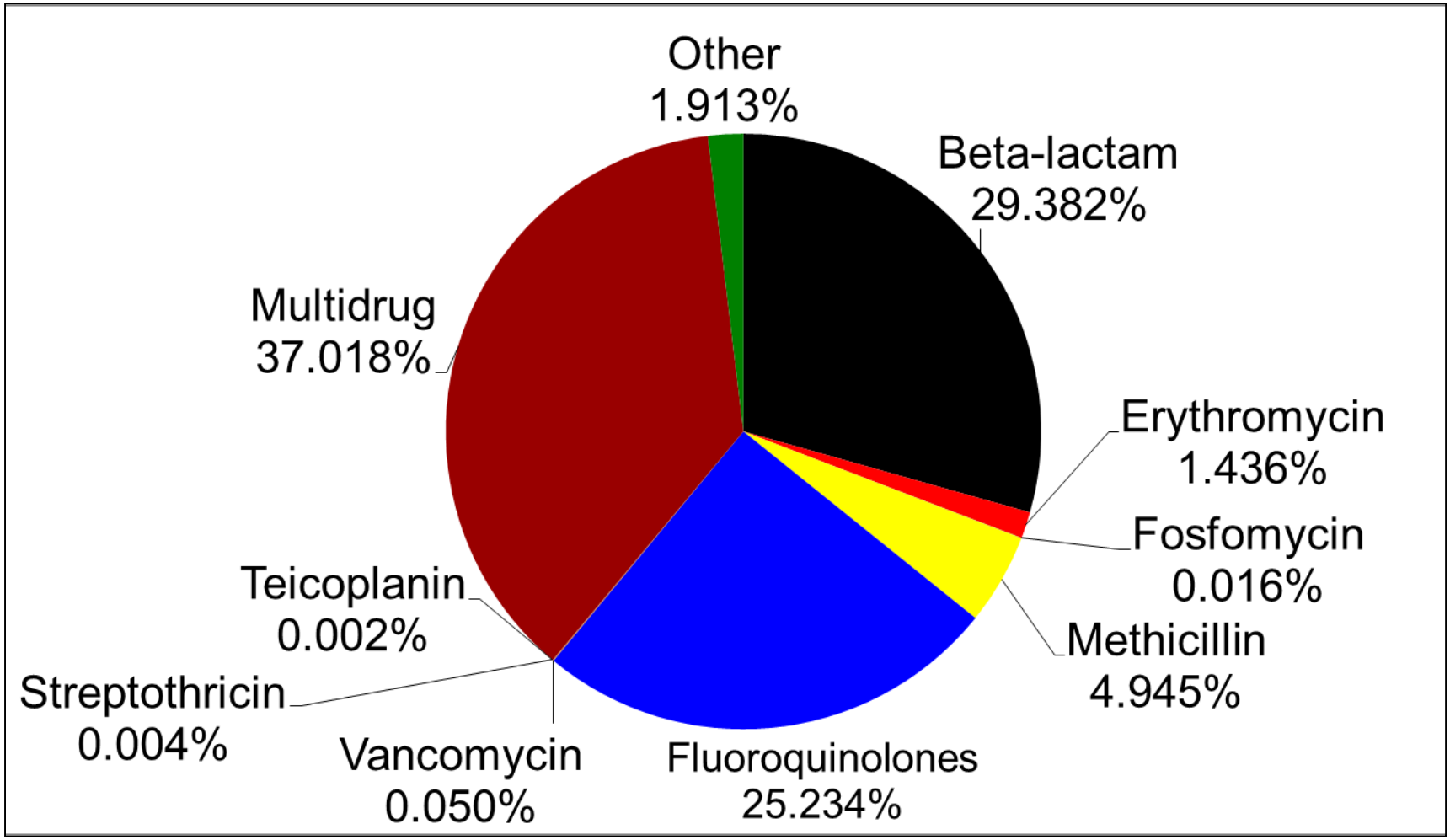




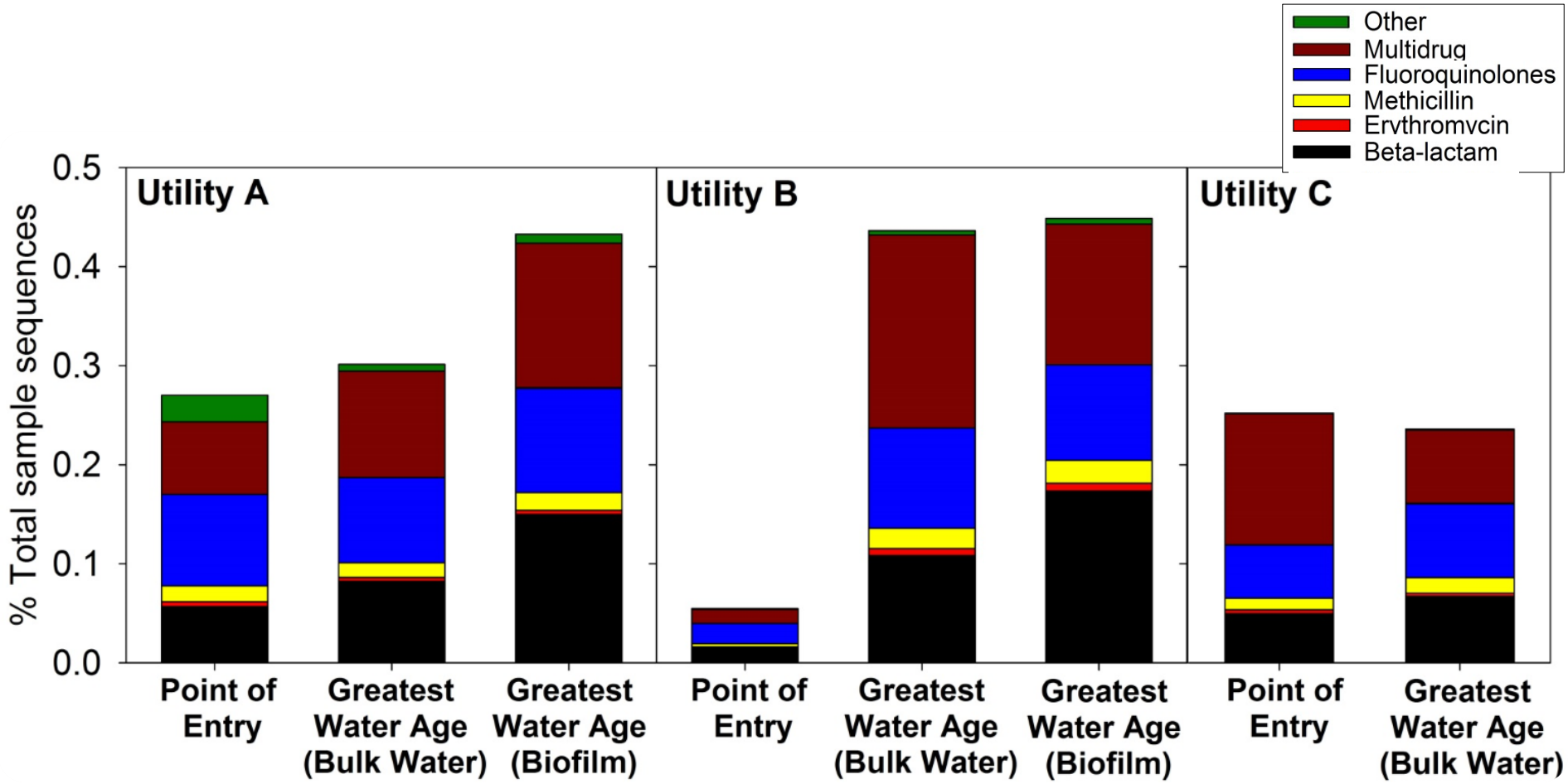
Microbial Community: Illumina Amplicon Sequencing



Metagenomics: Average ARG Composition



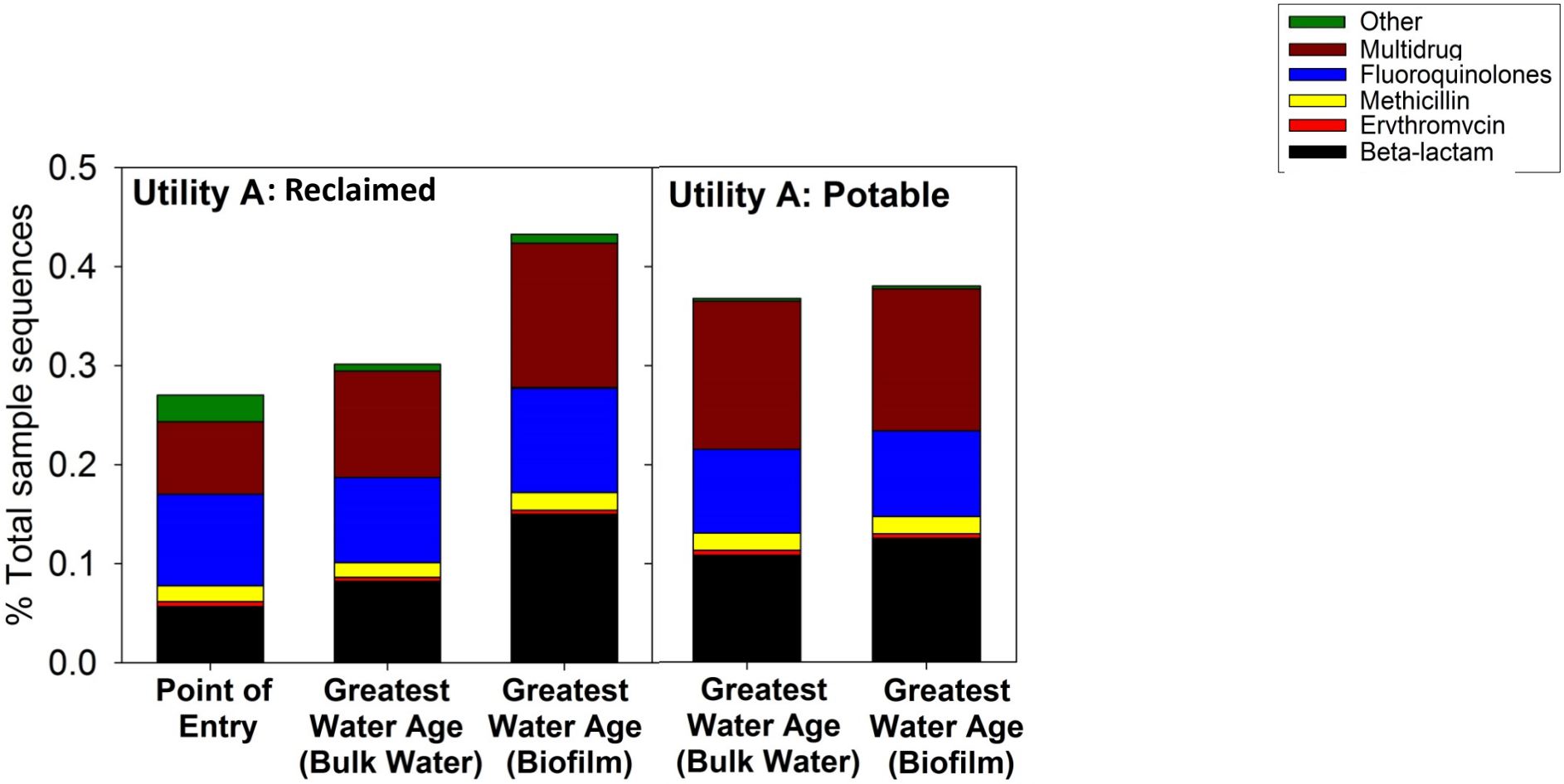
Metagenomics: ARGs in Reclaimed Water



Water Environment Federation
the water quality people®

WERF
Water Environment Research Foundation
Collaboration. Innovation. Results.

Metagenomics: ARGs in Reclaimed Water vs. Potable Water



Water Environment Federation
the water quality people®

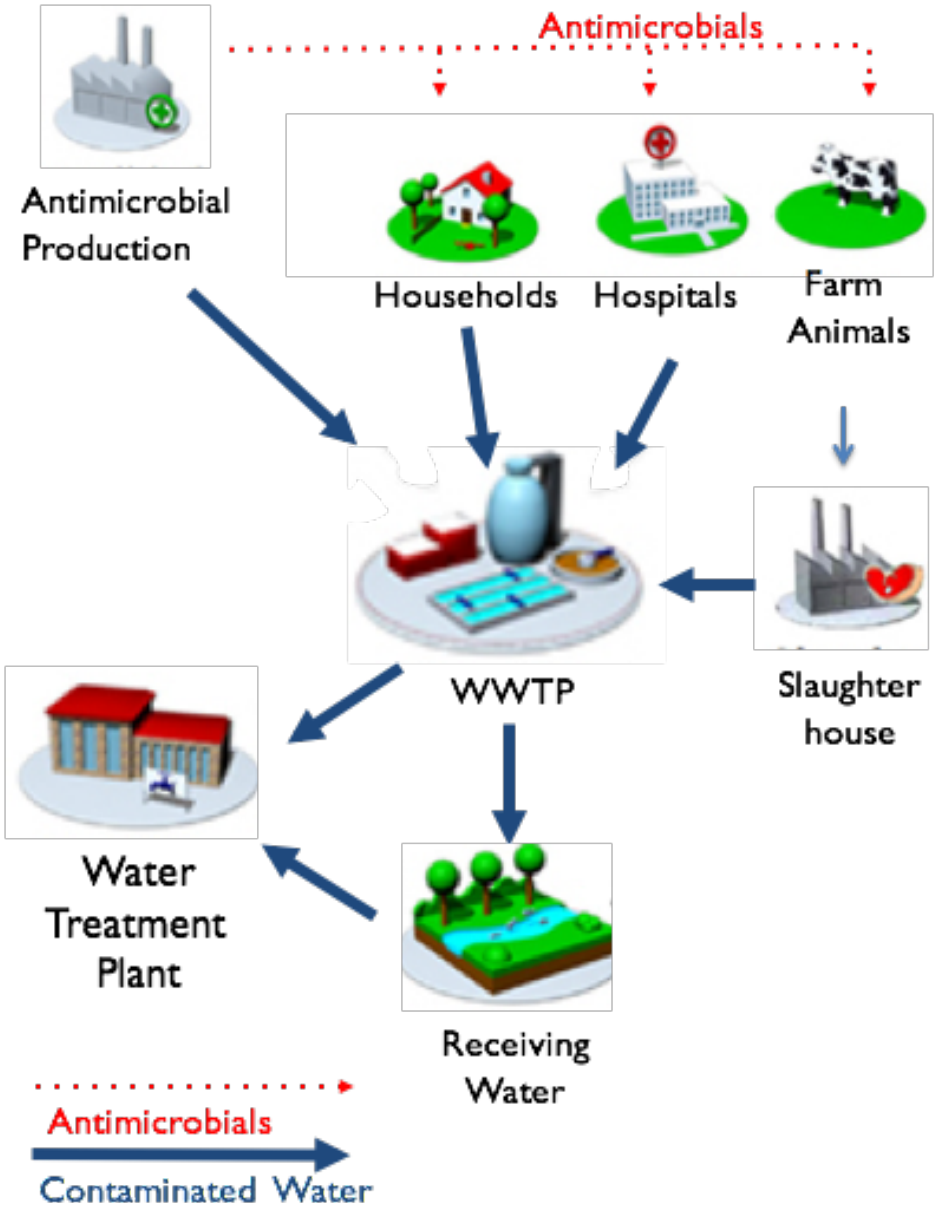
WERF
Water Environment Research Foundation
Collaboration. Innovation. Results.

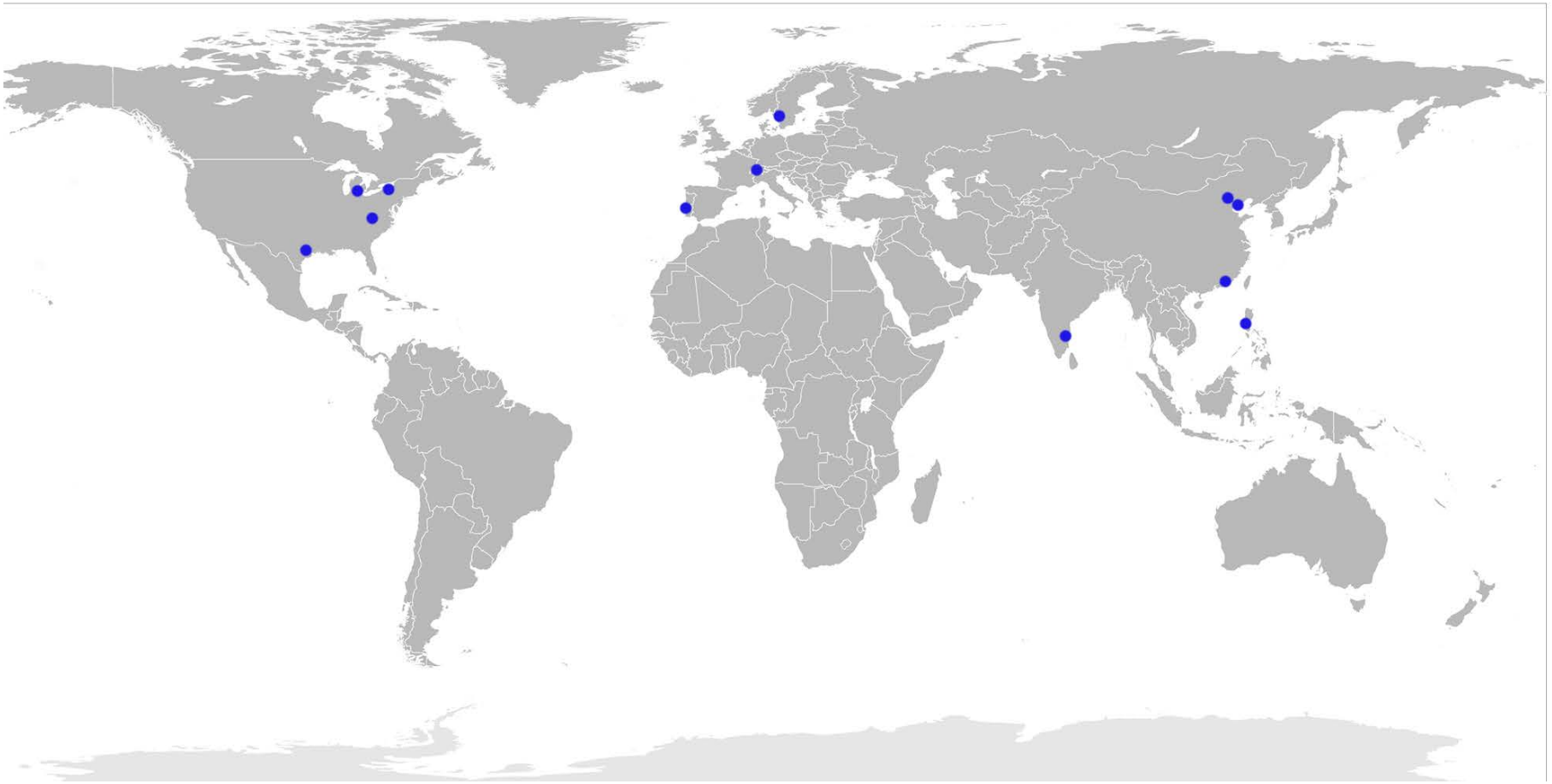
Halting Environmental Antimicrobial Resistance Dissemination

National Science Foundation

Partnership for International Research and Education (PIRE)

Halting Environmental Antimicrobial Resistance Dissemination (HEARD)





Peter Vikesland, Amy Pruden, Sridhar Venkataramana- Virginia Tech
Diana Aga- University at Buffalo
Pedro Alvarez and Qilin Li- Rice University
Krista Wigginton- U Michigan

5 yr, $3.6M

Environmental Science & Technology

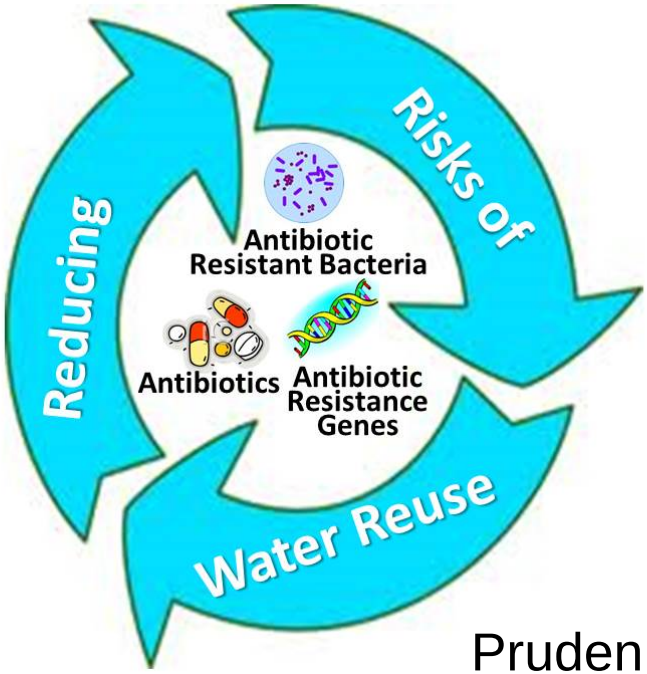
Feature

pubs.acs.org/est

Balancing Water Sustainability and Public Health Goals in the Face of Growing Concerns about Antibiotic Resistance

Amy Pruden[1,*]

[1]Via Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, United States



the United States and other developed countries, our water infrastructure has reached its design lifespan, as evidenced by the American Society of Civil Engineers combined grade of "D" for the U.S. drinking water and wastewater infrastructure.[5] Thus, we as a society face a key moment in history where we will either proactively take on the challenge of sustainable water infrastructure, or generally continue a much costlier reactionary approach. Clearly there is need for innovation, both technological and institutional, as recently reviewed by Kiparsky and colleagues.[6]

Initiatives, such as ReNUWit, a National Science Foundation Engineering Research Center lead by Stanford University and partners[7] challenge us to envision the city of the future in

Pruden, *ES&T* 2014 Editor's Choice: Best Feature Article

Who do we have to thank for our quality of life??

Doctors?

To some extent….

But really have to thank our plumbers and engineers!!

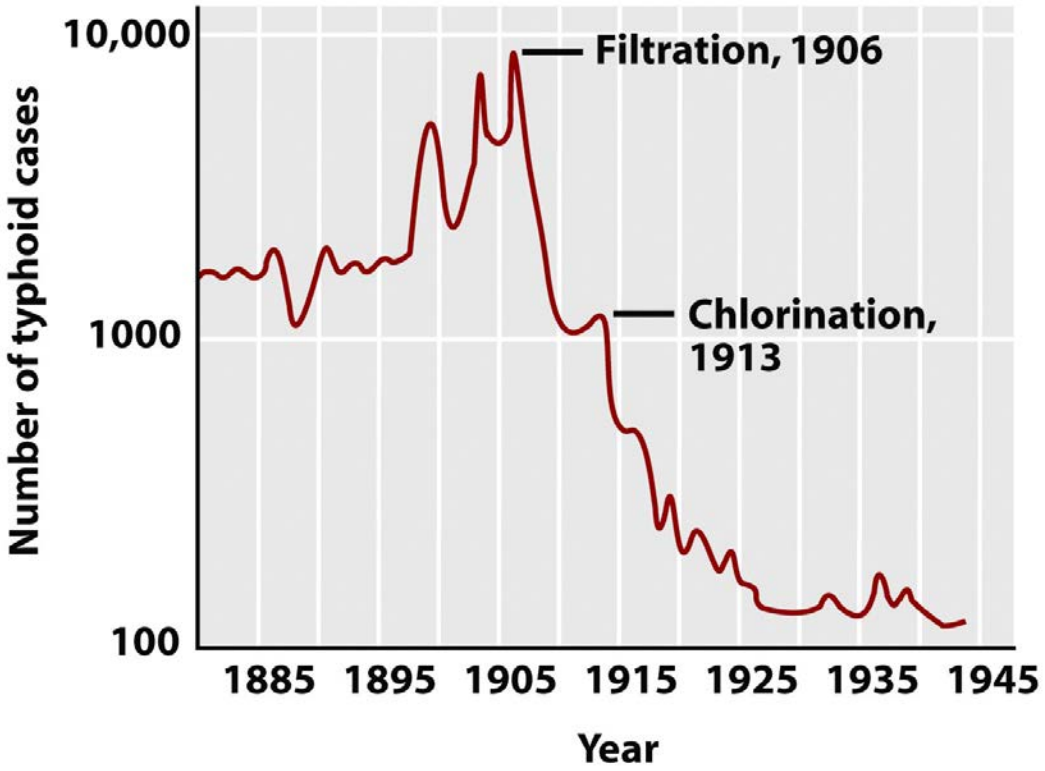
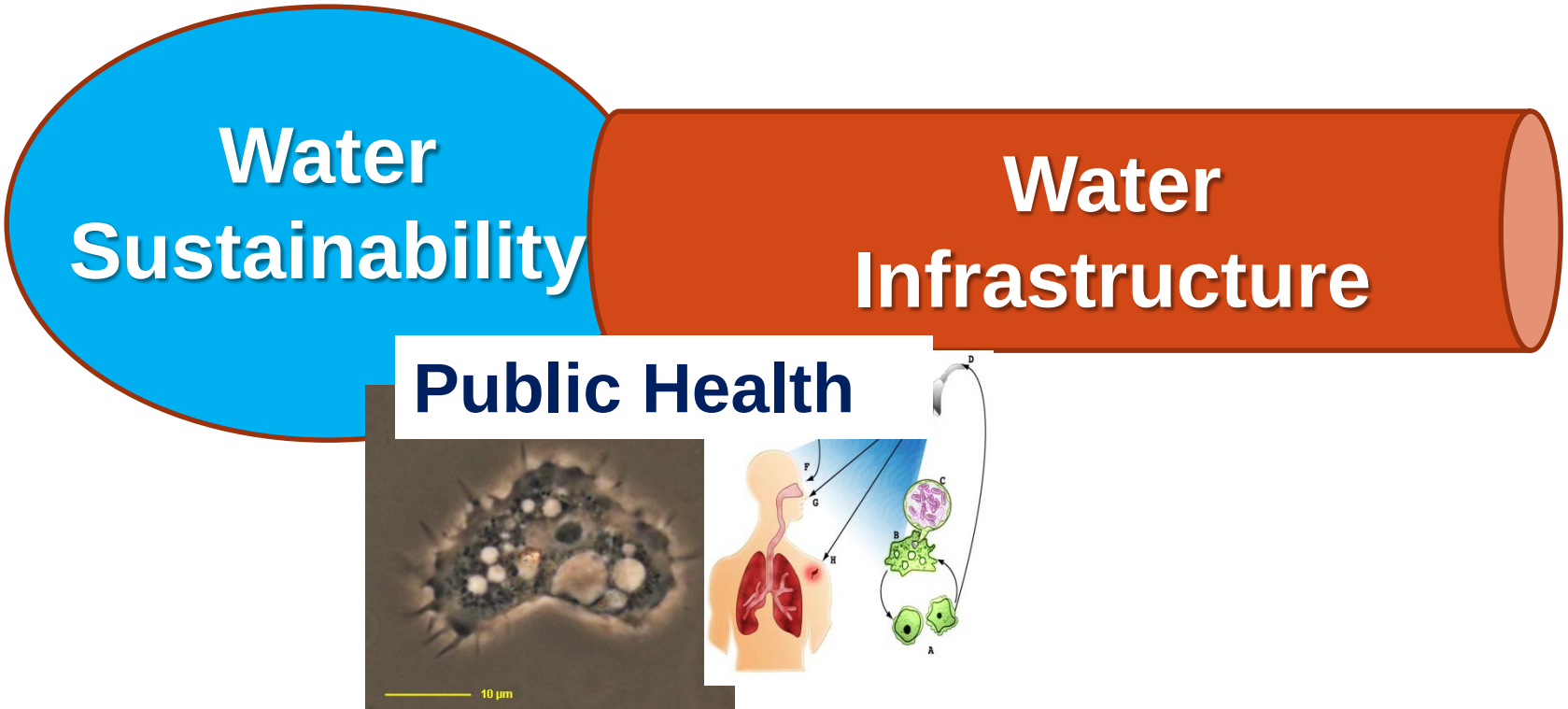


Figure 28-2 Brock Biology of Microorganisms 11/e

Take Home

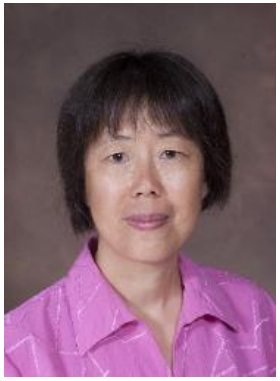
- Let’s do infrastructure right! Make informed decisions and invest as wisely as possible!



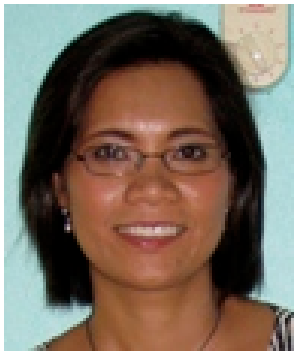
Leigh Anne Krometis, VTech

Cully Hession, VTech

Mazdak Arabi, CSU



Kang Xia, VTech



Diana Aga, U at Buffalo

Ken Carlson, CSU

Analytical Chemistry

Biological Systems Engineering/Watersheds

Marc Edwards, VTech

Peter Vikesland, VTech

Water Chemistry, Pipes, Sensors

John Novak, VTech

Wastewater Engineering

Jeannie McLain, U AZ

Jessica Davis, CSU

Katharine Knowlton, VTech

Agriculture, Livestock, Soil

- **Funding:** Alfred P. Sloan Foundation; National Science Foundation; Water Research Foundation, Water Environment Research Foundation

- **Students:** Nicole Fahrenfeld, Yanjun Ma, Heather Storteboom, Brittany Willing, Lindsey Caudle, Emily Garner, Caitlin Wilkinson, Partha Ray, Heather Littier, Jennifer Miller

Thank You!