

The Impact of Wastewater on the Development of Antibiotic Resistance in the Environment

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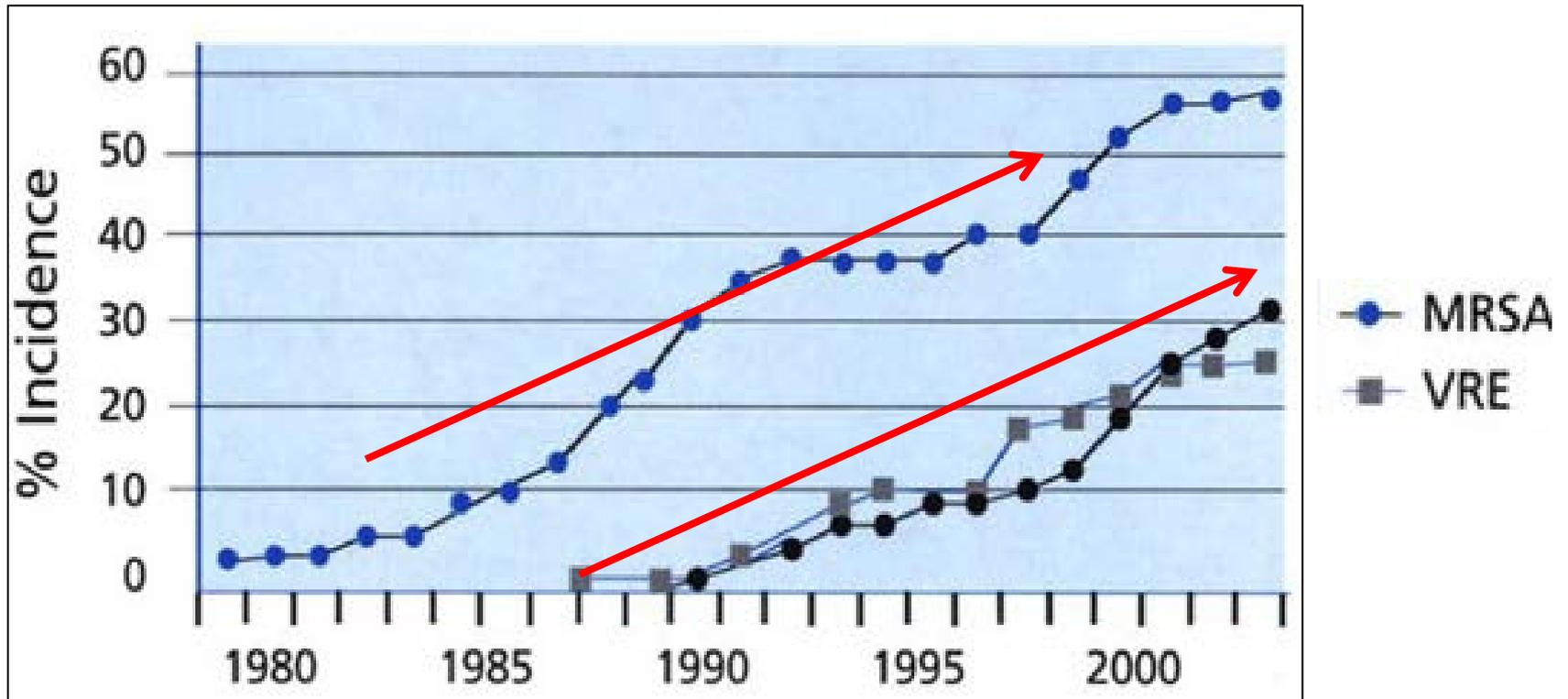
Antibiotic Resistance (AR)

- World Health Organization: “one of the most critical human health challenges of the next century”
- WWTPs not designed for the removal of these micropollutants
- Low levels of residual antibiotics in the WTTP may promote selective growth of resistant microorganisms
- Proposed that this is a “key source of resistance into the environment”



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

MRSA and VRE on the Rise



notunlikeresearch.typepad.com

MRSA = Methicillin-Resistant Staphylococcus Aureus

VRE = Vancomycin-Resistant Enterococci

AR in the Media

New superbug found in two patients here

By POON CHIAN HUI
A NEWLY found superbug from India that has been making news around the world infected two patients here early this year - before anyone knew what they were dealing with - but was successfully dealt with and contained.

The Ministry of Health (MOH) told The Straits Times that the patients had infections from bacteria with the New Delhi metallo-beta-lactamase-1 (NDM-1) gene identified last month.

The gene has the ability to shield bacteria from all antibiotics, turning them into drug-resistant superbugs.

It was found in the samples from the two patients last month, after hospitals went back and tested past samples.

"Following reports of NDM-1 in other countries and the availability of newer and more sensitive tests, our hospitals found two cases from the beginning of this year that were positive for NDM-1," said an MOH spokesman.

One was a Singapore resident who had sought medical treatment in India, while the other had come to Singapore from Bangladesh for medical treatment.

They had been admitted at different times to the Singapore General Hospital. The patients were screened for drug-

resistant bacteria signs of illness but gone so far - as the two patients were not infected with both were quick patients after bacteria were found in bed rooms and lavatories.

Health-care staff there also donned gowns and gloves.

Both patients underwent antibiotic treatment subsequent to the growth of the bacteria. It is not known they were given,

Four more infected by new superbug

They are likely to have caught the bug locally; more cases may surface

By SALMA KHALIL
HEALTH CORRESPONDENT

ANOTHER four people in Singapore have been infected by the new superbug, the highly drug-resistant New Delhi metallo-beta-lactamase 1 (NDM-1), bringing the total to six.

Of the latest cases, three were elderly patients aged between 74 and 84 but the fourth was in his 30s. As some of them had travelled abroad, it is likely that they had caught the bug locally.

The youngest patient may have acquired the superbug while in hospital as he developed a urinary tract infection af-

ter a two-month stay in the United States.

Health Minister Mr Khaw told the NDM-1 "is a species and strain first detected in December last year."

NDM-1 has been countries because are difficult to treat.

An article in the latest issue of the journal has potential of NDM-1 as a public health problem.

02 SINGAPORE HEALTH MAY/JUN 2013

News

The rise of the superbug

SGH's Director of Infection Control clues to the rise of the superbug
of simple hygiene habits to combat the superbug



BY JACQUELINE CHIA

HE WOKE UP COUGHING, FEELING WEAK AND WITH BODY ACHES. JUST THE FLU, he thought.

science Conference on Antimicrobial Agents and Chemotherapy urged the health authorities to track bacteria with the gene.

The conference in the United States, which ended yesterday, is the world's largest gathering of infectious disease specialists, attracting about 12,000 people.

Drug-resistant superbugs are not new and include methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*.

NDM-1 is a serious infection found

theguardian

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News > Society > Antibiotics

Antibiotic-resistant diseases pose 'apocalyptic' threat, top expert says

Chief medical officer Dame Sally Davies tells MPs issue should be added to national risk register of civil emergencies

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Ian Sample, science correspondent
The Guardian, Wednesday 23 January 2013 14.41 EST
Jump to comments (503)

Article history

Society
Antibiotics · Drug resistance · Health

UK news

Politics
Health policy

Science
Drugs · Infectious diseases · Microbiology · Medical research · Biology

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Local Attention

Flagstaff weighs action on resistant bacteria find

Posted: Apr 08, 2013 11:18 AM

Updated: Apr 22, 2013 11:18 AM

Posted by Dawn Alexander - [email](#)

NEW STUDY FINDS ANTIBIOTIC-RESISTANT BACTERIA GENES IN FLAGSTAFF'S RECLAIMED WATER

Posted By [Linda Dailey Paulson](#) On Sep 19, 2012 In [Water In America](#), [Water Research & Reports](#)

October 11, 2012

Wastewater Snow-Making 'Could Breed Super-Bacteria'

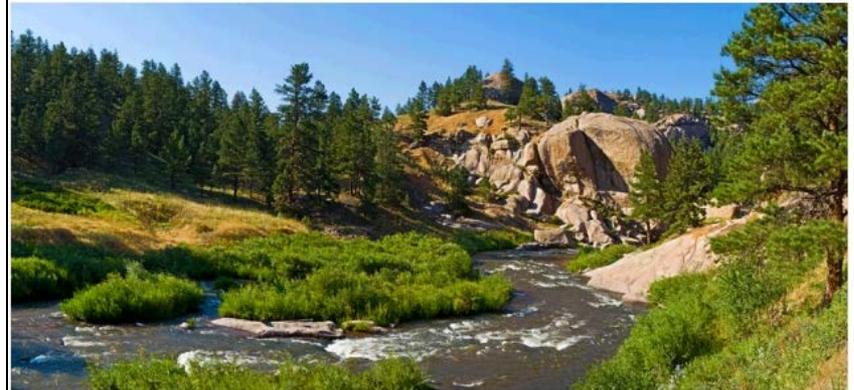
NATURE | NEWS

Antibiotic resistance racing downriver

A river that runs through Colorado's plains carries two different genes that protect microbes from antibiotics.

Naomi Lubick

17 October 2012



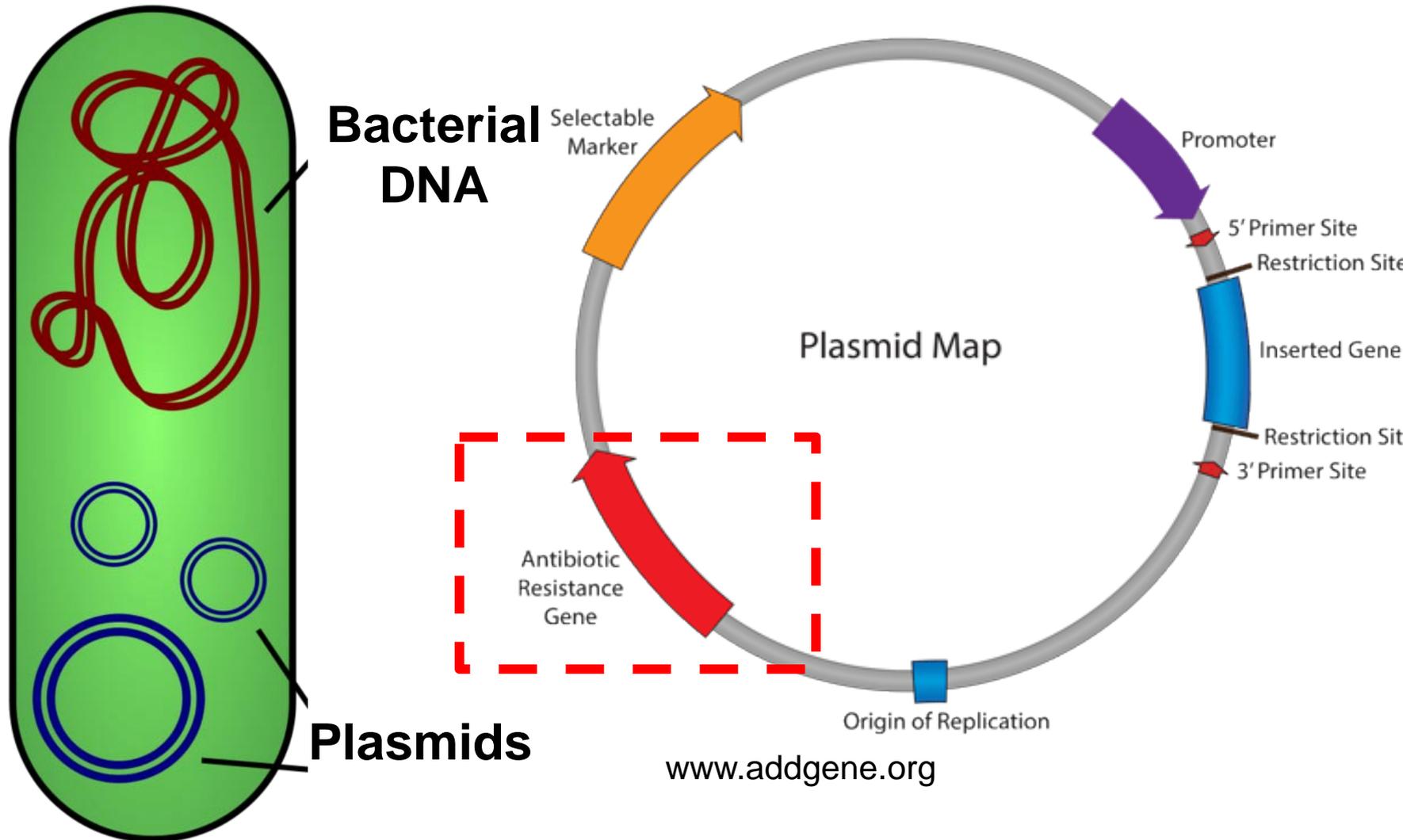
The pristine Platte river picks up microbial genes for antibiotic resistance as it flows past human settlements.

PHILIP NEALEY/GETTY

What is antibiotic resistance?

- Antibiotics
- Antibiotic Resistant Bacteria
- Antibiotic Resistant Genes

Antibiotic Resistance Genes (ARG) and AR Bacteria

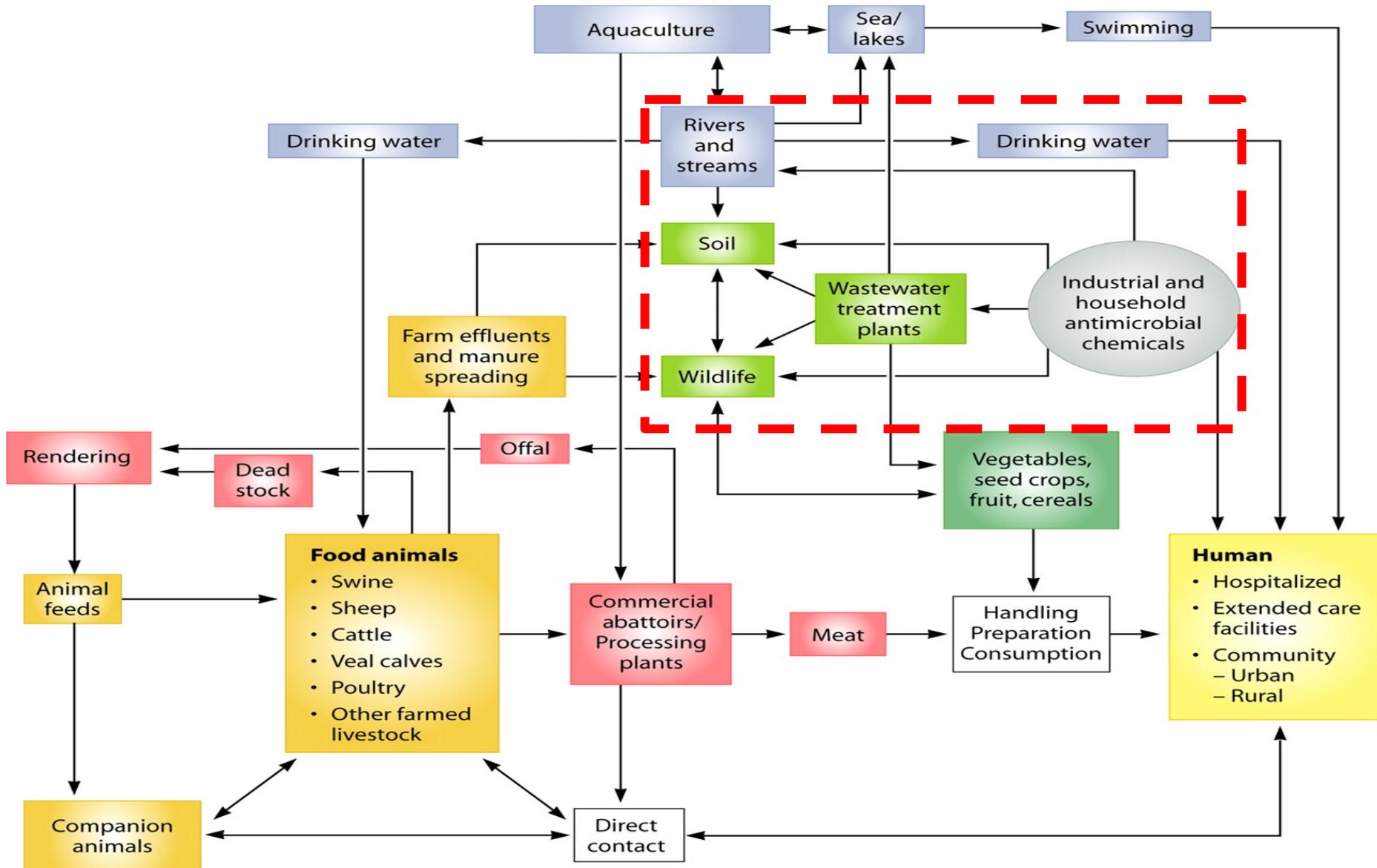


It is important to remember...

- Antibiotics were first isolated from the environment.
- Antibiotic resistance is a natural phenomena that allows for bacteria to compete (or out compete) for resources.



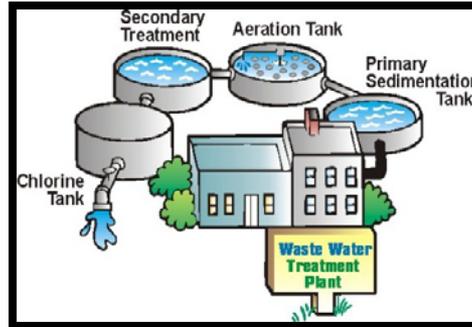
Dissemination of Antibiotics and Antibiotics Resistance



Shifting Research Objectives



Multi-millions of antibiotics prescribed daily



Wastewater treatment plants discharge treated water into the environment



Can treated wastewater transport antibiotic resistant bacteria and their resistance genes?



Optimization of the treatment process may lower the spread of antibiotic resistance throughout the environment.

Are AR bacteria a concern for water reuse?

- New emerging contaminant?
- Are current treatment technologies sufficient for their removal/disinfection?
- Can they confer resistance to native bacteria in the environment?
- Human health impacts? Risk assessment?

Current Research

- Several studies have identified ARG's in secondary and tertiary treated recycled water.
- Genes have also been found in the environment downstream of surface water discharge locations.
- AR bacteria have been found in biosolids, land application sites, recharge ponds, retention basins, etc.



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Temporal dynamics of antibiotics in wastewater treatment plant influent

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Laboratoire de Technologie Écologique,
Ecole Polytechnique Fédérale de Lausanne

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1

The scourge of antibiotic resistance: the important role of the environment

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Peter Collignon, Infectious Diseases and Canberra Clinical School, Australian National University, Canberra, Australia

D. G. Joakim Larsson, Department of Infectious Diseases, Sahlgrenska University Hospital, of Gothenburg, Göteborg, Sweden

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Effect of River Landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG)

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Department of Civil & Environmental Engineering, 1372 Campus Mail, Colorado State University, Fort Collins, CO 80523, USA

ARTICLE INFO

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ABSTRACT

The purpose of this study was to quantify antibiotic resistance genes (ARG) in the sediments of the mixed landscape Cache La Poudre River, which has previously been

AR in WWTPS

- “Survival of MRSA strains in UHWWs and their transit to the STP and then through to the final treated effluent and chlorination stage.”

- Thompson et al. 2012

- “Urban wastewater treatment plants (UWTPs) are among the main sources of antibiotics' release into the environment”

–Rizzo et al., 2013

- “ARB and antibiotic resistant genes (ARGs) have been detected extensively in wastewater samples.”

-Bouki et al. 2013

Effect of Solids Retention Time (SRT)

- Though **increased** solids retention time (SRT) has been correlated with **reductions** in trace antibiotics, higher SRTs also provide prolonged exposure of bacteria to influent antibiotic levels, potentially increasing the development of antibiotic resistance

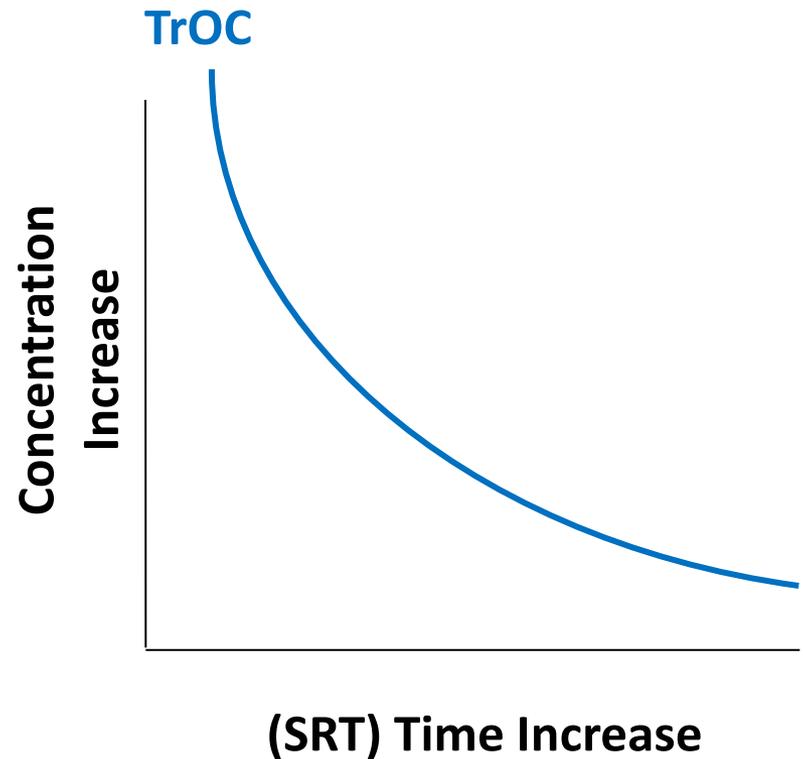


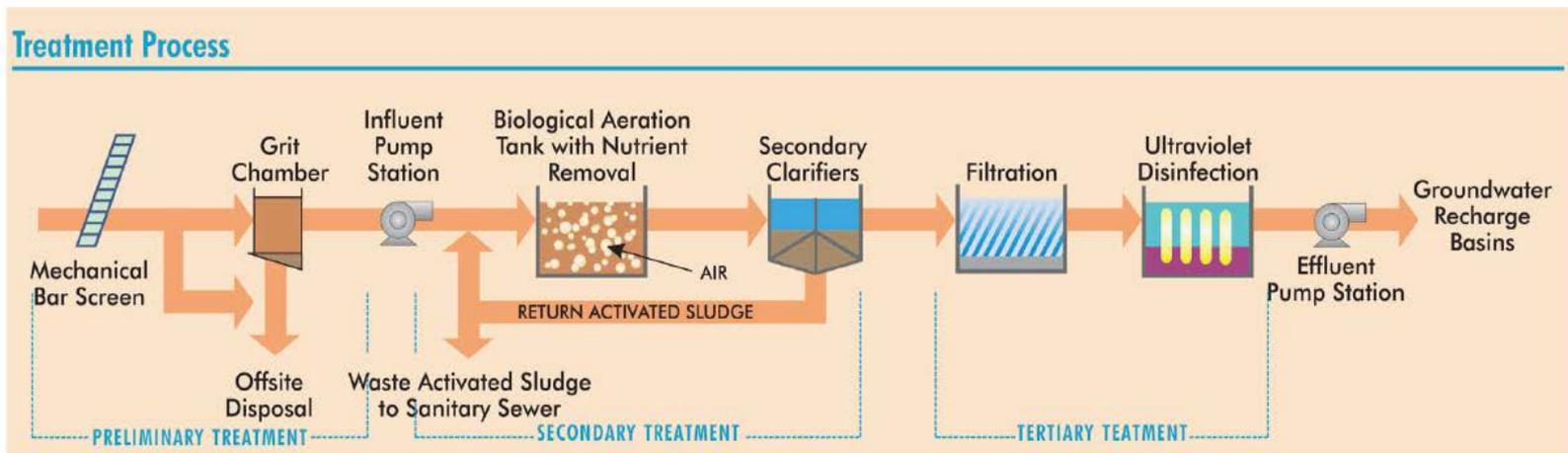
Table 1. Threshold SRT 80% Degradation^Φ

TrOC	SRT (Days)
Acetaminophin	2
Caffeine	2
Ibuprofen	5
Naprozen	5
Bisphenol A	10
Triclosan	10
DEET	15
Gemfibrozil	15
Atenolol	15
BHA	15
Iopomide	15
Cimetidine	15
Diphenhydramine	20
Benzophenone	20
Trimethoprim	30

^Φ Trace Organic Compound Removal During Wastewater Treatment, IAT Report, November 2011.

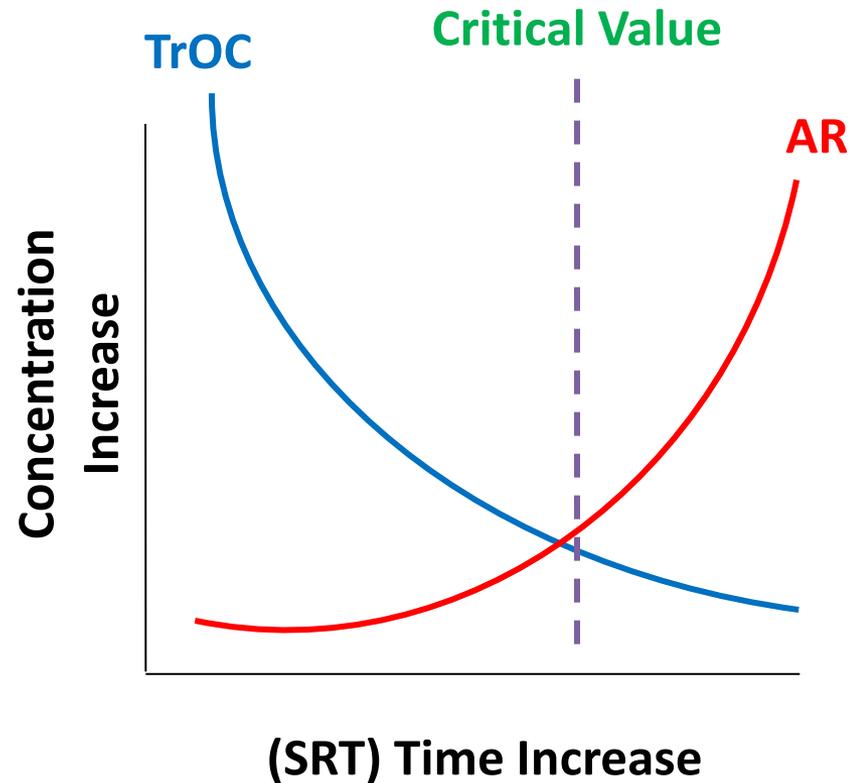
Solids Retention Time (SRT)

- Conventional Activated Sludge (CAS)
- Exposes bacteria to ideal growth conditions
- SRT indicates the mean residence time of microbes in the reactor
- High SRT allow the enrichment of slowly growing bacteria; physiological diversity



Effect of Solids Retention Time (SRT)

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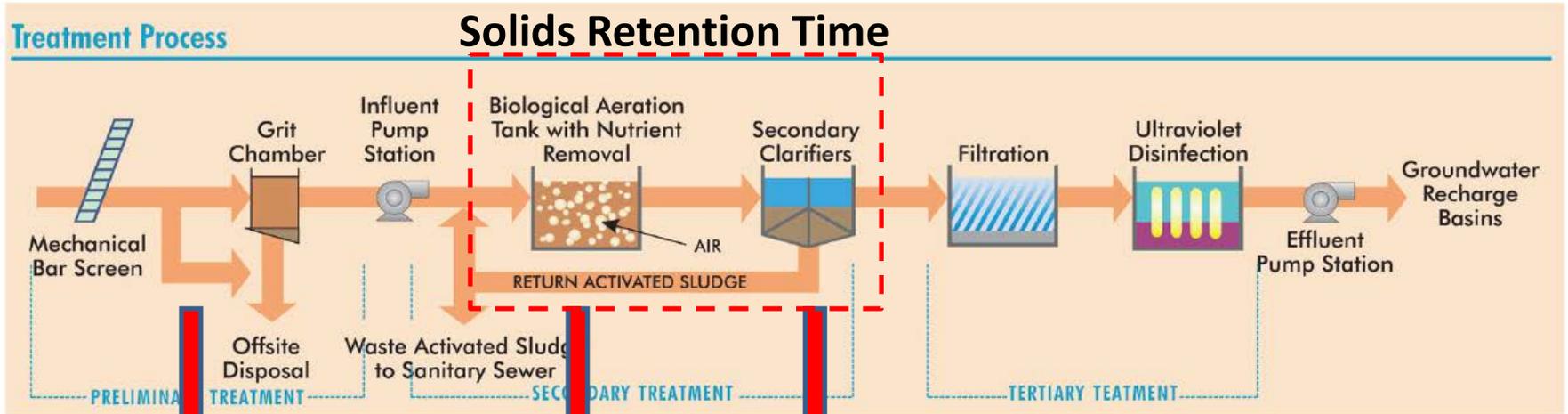
Research Question

Can the wastewater treatment process be optimized to mitigate the presence and persistence of antibiotics and antibiotic resistant bacteria?

Selected Solids Retention Times

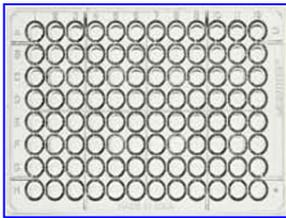
WWTP Site	SRT (days)
Plant 1	<1
Plant 2	1.5
Plant 3	3
Plant 4	9
Plant 5	12
Plant 6	14
Plant 7	19
Plant 8	45

Materials and Methods

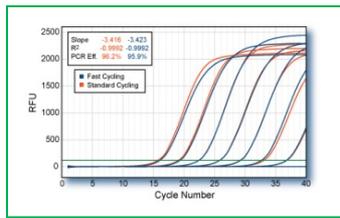


Molecular & Cultural Analysis

Chemical Analysis



Bacterial Isolates (MIC)



Quantitative PCR



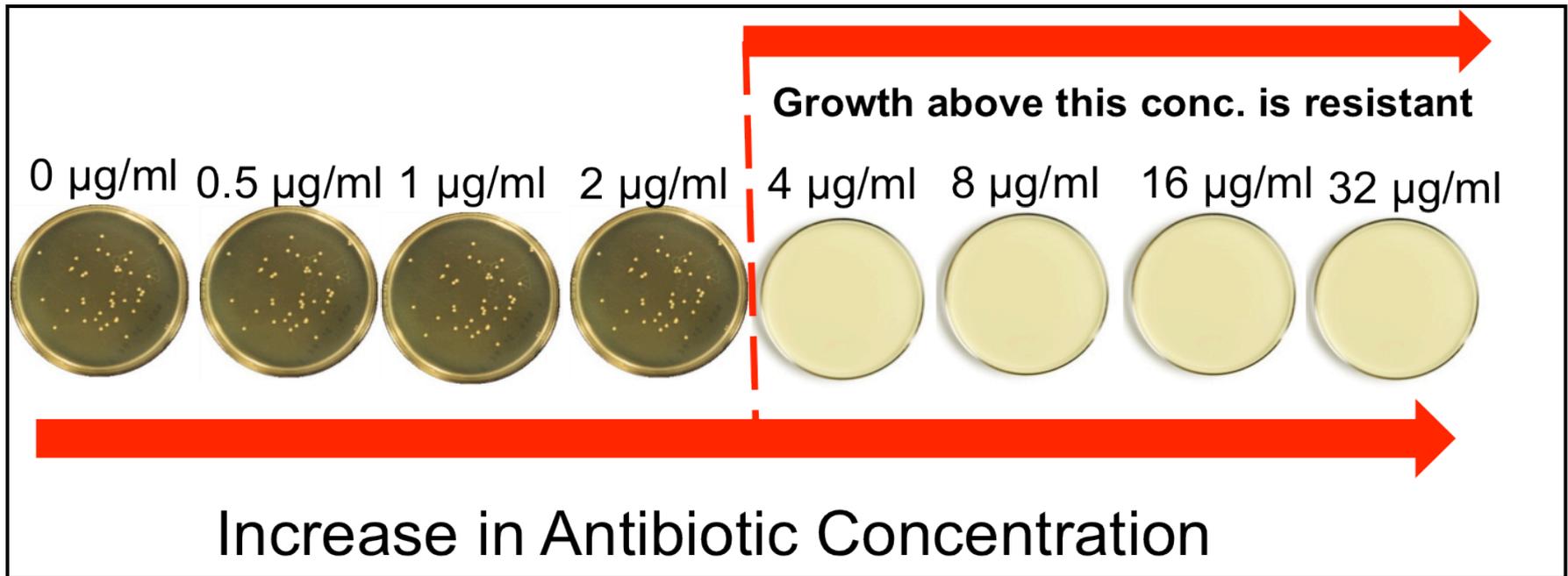
SPE



LC-MS/MS

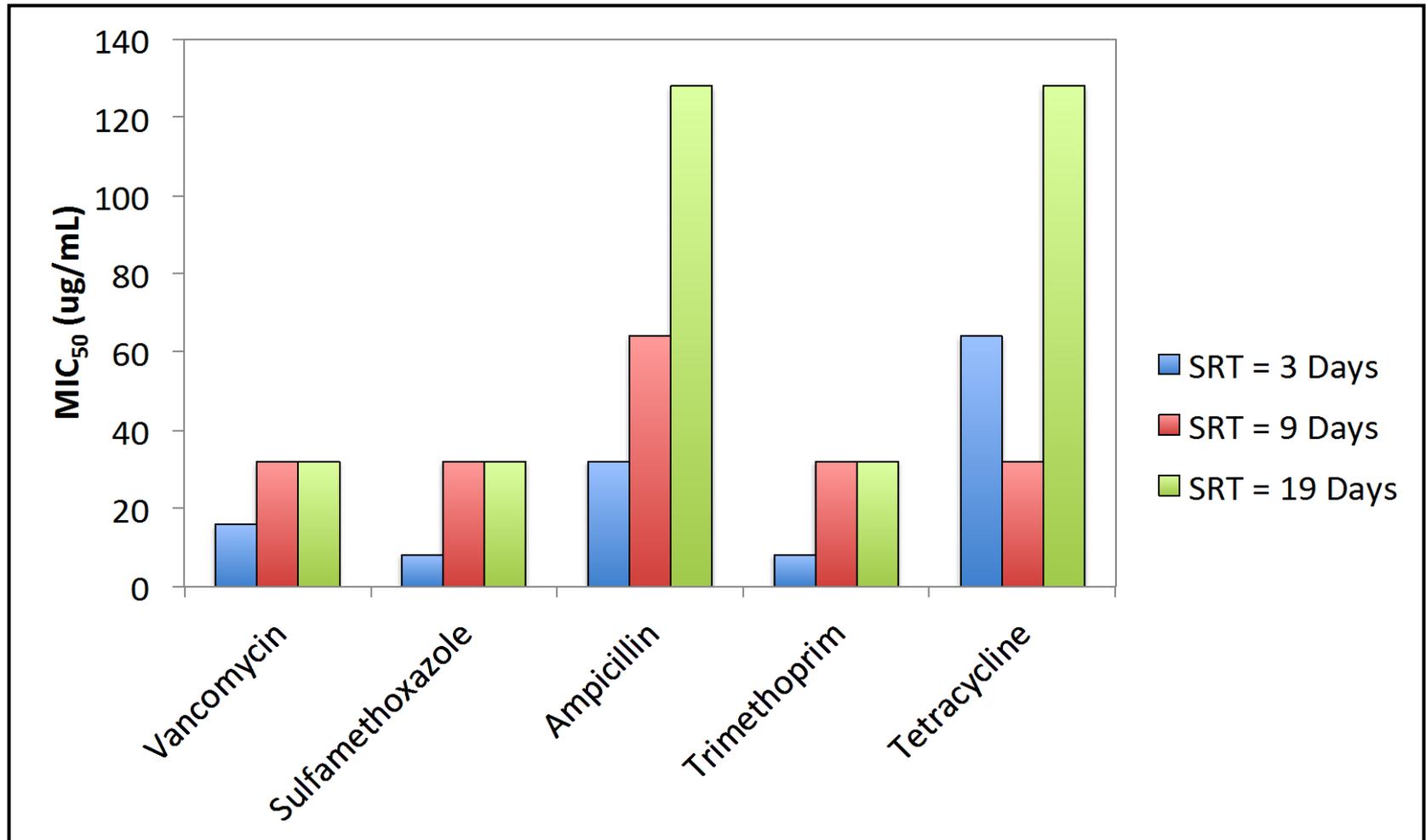


How Resistant?



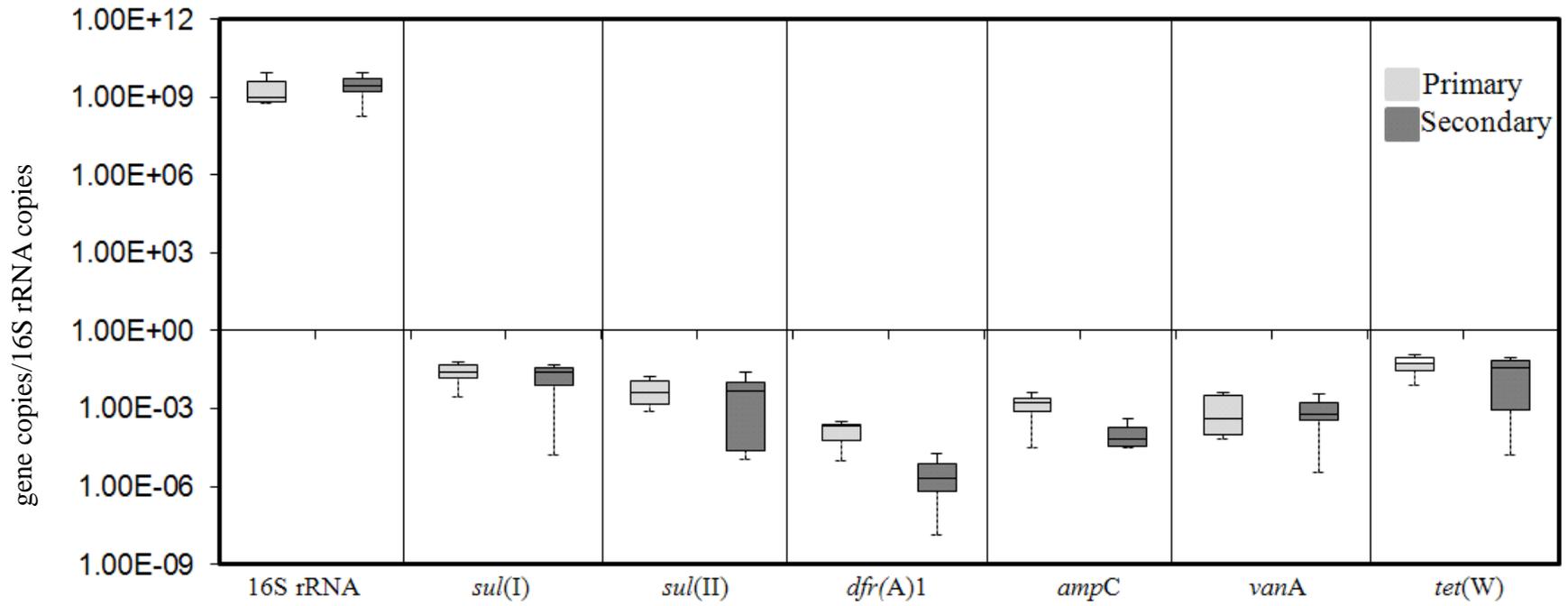
- **MIC₅₀** is a way of recording antibiotic sensitivities more conveniently, especially if your trying to report MICs of a large group or organisms.
- **MIC₅₀** is the concentration required to inhibit the growth of 50% of organism

Increase in Antibiotic Resistance



(MIC₅₀ is the concentration required to inhibit the growth of 50% of organisms)

Antibiotic Resistance Genes



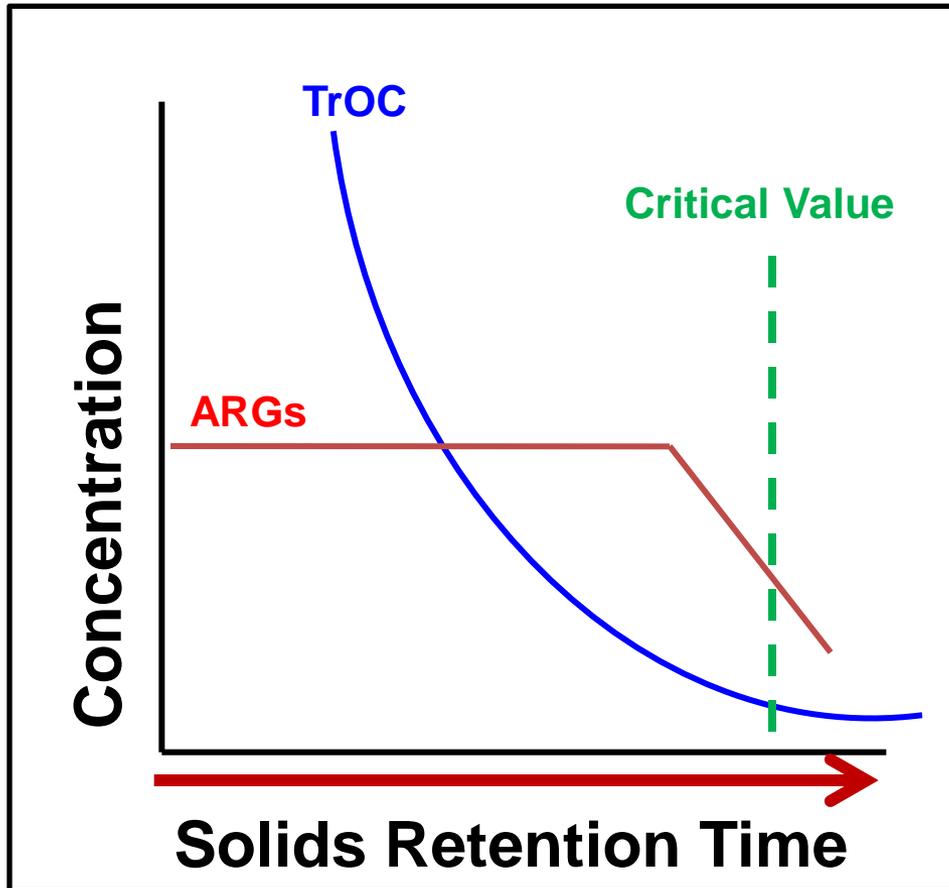
Research Findings

- Our lab developed standardized cultural, molecular, and analytical-based approaches that provided a means to survey wastewater for the occurrence of AR
- All six ARGs were abundant throughout the treatment train of all eight WWTPs that confer resistance to clinically important antibiotics
- The relative abundance of different ARGs showed significant variations among removal efficiencies during the biological treatment processes (i.e. SRT)

Research Findings cont.

- High SRT allow the enrichment of slowly growing bacteria; physiological diversity
- Non-AR bacteria are outcompeting AR-bacteria
- TOrCs compounds may enhance the development of AR; increased MIC₅₀
- AR bacteria that are present at high SRTs may develop an advanced tolerance to antibiotics

Outcome



- Varying solids retention times appear to have an effect in mitigating proliferation of antibiotic resistance.
- But what is the **impact/relevance** of these viable microbes, inactivated microbes, and genes in the **environment** and with respect to **human health**?

Future Research

- Are there seasonal variations in the loading and performance of treatment on the prevalence/ development of AR?
- Detailed research, including mass balance of resistance genes, needed to assess true potential for spread of antibiotic resistance.
- What are the risks associated with ARGs leaving WWTPs?
- What happens once ARGs are released into the environment?
- Does exposure to antibiotic resistance genes have negative impact on environmental/human health?

WHY SHOULD I CARE?

How Did ABR Become Connected to Recycled Water Use?

The Case of Flagstaff, Arizona

Aug 13: Antibiotic resistance genes
in pipe outflows of recycled water
system



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The New York Times

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The New York Times

The Washington Post

How Did ABR Become Connected to Recycled Water Use? The Case of Flagstaff, Arizona

December 2012: First meeting of Flagstaff City Manager's Expert Panel on Antibiotic Resistance



How Did ABR Become Connected to Recycled Water Use?

The Case of Flagstaff, Arizona

December 2012: First meeting of Flagstaff City Manager's Expert Panel on Antibiotic Resistance

Panel Agrees:

1. Antibiotic resistance genes \neq resistant bacteria (DNA can indicate living or dead)
2. Recycled water pipe only: what about drinking/potable water pipes?



WHAT COMES NEXT?

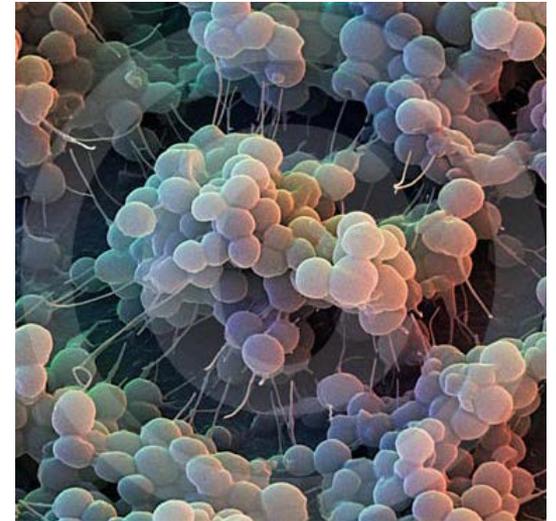
What do we NOT know?

- Does environmental presence of antibiotics and antibiotic resistance genes = increased resistance?
- If background levels of resistance are ubiquitous in the environment, how can we measure an increase?
- Has this background resistance skewed research results?



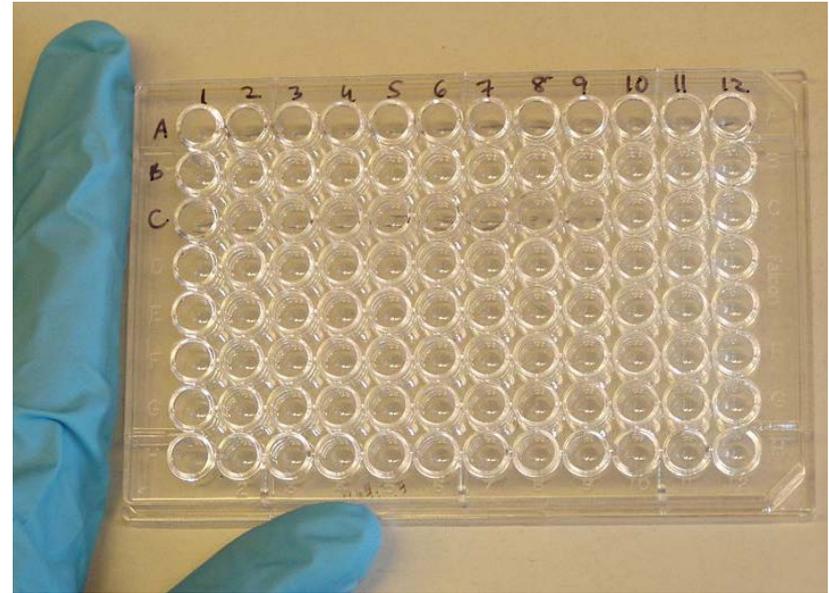
Peer-Reviewed Publications of Environmental Antibiotic Resistance (2012-2013)

- 200 Papers with Web of Science search “**Antibiotic Resistance**” and “**Agriculture**” or “**Environment**”
- 72% utilized molecular methods
 - 67 genes
- 14% utilized cultivation
 - *Enterococcus*
 - *E. coli*
- 14% utilized combination



Resistance Analysis Methods: Bacterial Isolation/Culturing

- Broth micro-dilution methods
- Agar disc diffusion
- Clinical and Laboratory Standards Institute

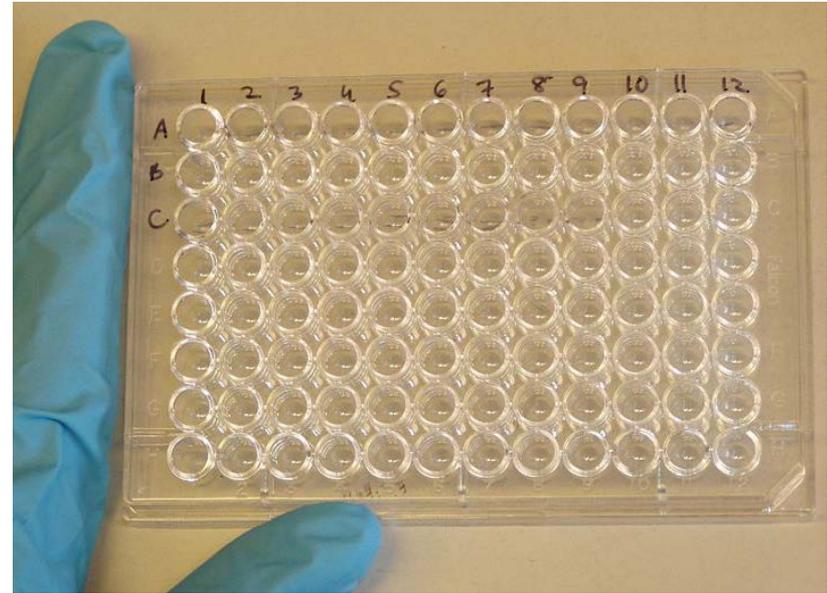


Resistance Analysis Methods: Bacterial Isolation/Culturing

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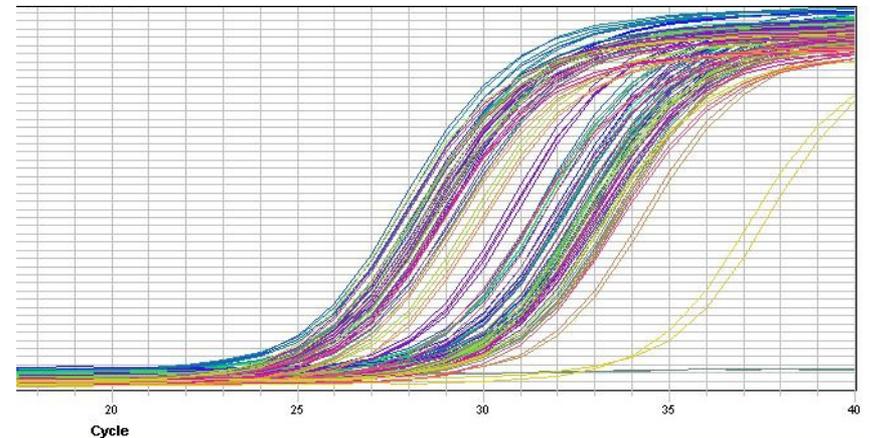
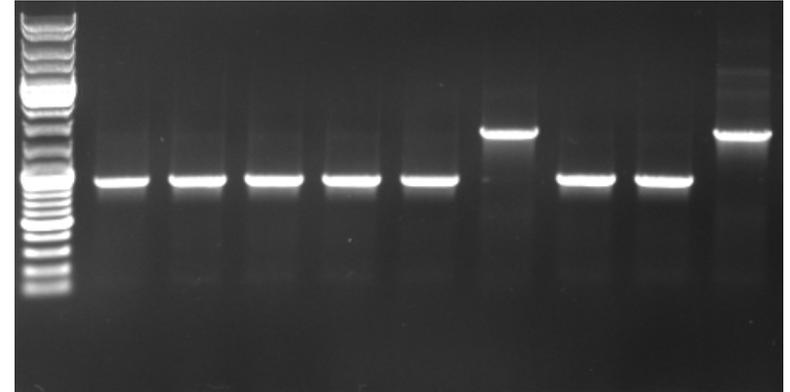
PROS: Highly reproducible, robust, Can assess at clinical levels

CONS: Analyze a single isolate in each plate



Resistance Analysis Methods: Molecular ID of Genes

- Polymerase Chain Reaction (PCR)
- Quantitative PCR (qPCR)



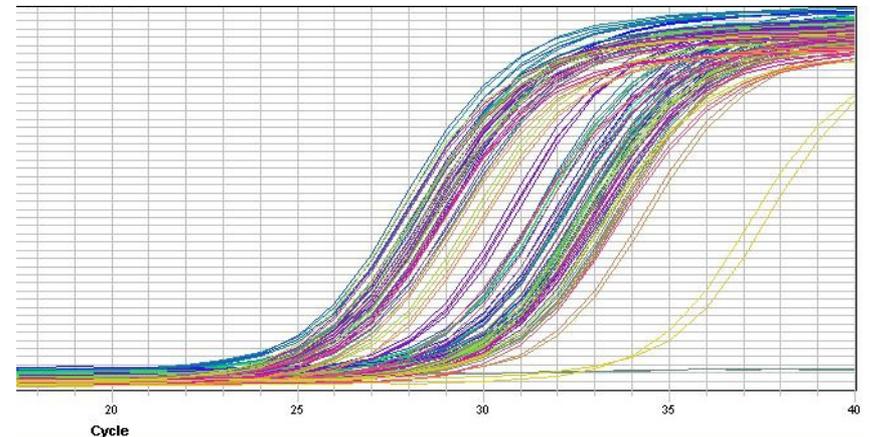
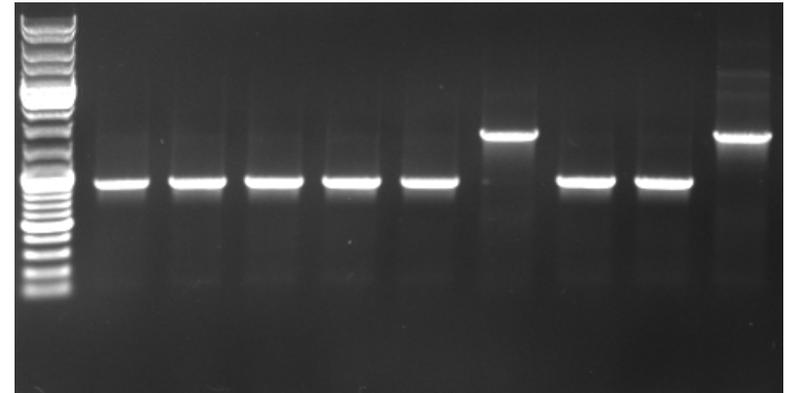
Resistance Analysis Methods: Molecular ID of Genes

- Polymerase Chain Reaction (PCR)
- Quantitative PCR (qPCR)

PROS: Can identify/quantify resistance genes in entire DNA sample, Ability to quantify to single gene marker level

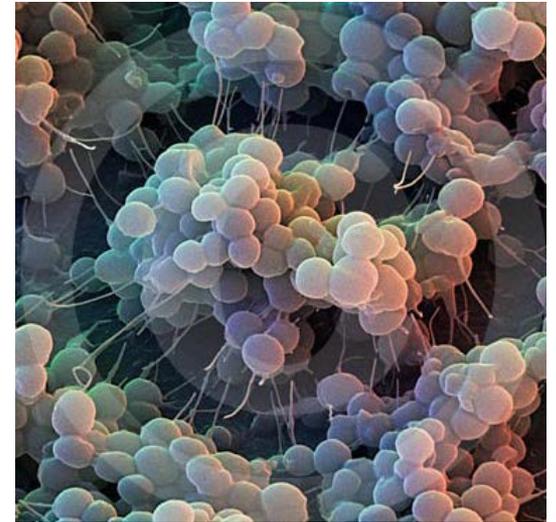
CONS:

No discrimination between live and dead bacteria



Peer-Reviewed Publications of Environmental Antibiotic Resistance (2012-2013)

- 43% lacked control samples from un-impacted sites
- 92% of papers lacking control sites reported increases in resistance from agricultural activities
- Papers with controls, 38% reported significant increases in resistance from agricultural activities



Antibiotic Resistance in the Environment: A Call to Action

- 2014 Meeting at Biosphere2 (McLain, Durso, Snow) funded by USDA-NIFA
- Recognizing that release of trace antibiotics and resistance genes is occurring, but that effects/risks are unknown
- Scientific community will draft proposed methods for assessing ABR in the environment



Antibiotic Resistance in the Environment: A Call to Action

Objectives:

1. To reach consensus on methodology
1. To train next generation in new methods
1. To address misinformation



Antibiotics in Agroecosystems: State of the Science

Organizers: University of Arizona
USDA-Agricultural Research Service
University of Nebraska

When: August 2014, Biosphere2, Oracle, Arizona

- A gathering of scientists focused on addressing the study of antibiotic resistance in an applied way.
- We contend that scientists need to step back and first address pre-existing, background resistance and natural antibiotic production and furthermore, must come to a consensus on methods used to assess antibiotic resistance in natural ecosystems.

Invited Experts: Amy Pruden, Ph.D. , Virginia Tech University (Blacksburg, VA).
Eddie Cytryn, Ph.D., State of Israel Agricultural Research Org.
Alistair Boxall, Ph.D. , University of York (U.K.),
Diana Aga, Ph.D. , State University of New York (SUNY) at Buffalo.

Biosphere2 Conference



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Lisa Durso – lisa.durso@ars.usda.gov

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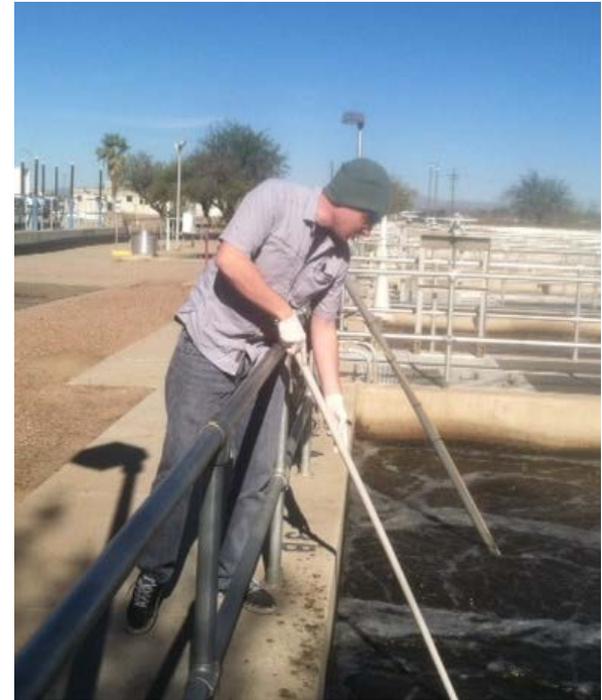
Dr. Daniel Gerrity

Dr. Leif Abrell

- Rachel Jo Maxwell

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