Seasonal Occurrence and Reduction of Viruses and Protozoa in Two Wastewater Reclamation Facilities in Southern Arizona

Masaaki Kitajima, Brandon C. Iker, Ian L. Pepper, Eiji Haramoto, and Charles P. Gerba
OBJECTIVES

- Evaluate wastewater treatment efficacy for virus and protozoa removal
- Look at seasonal effects
- Look for potential model virus indicator
Which will be most abundant:

- Virus that affects small segment of populations
e.g. adenovirus

- Or virus excreted by all members of community?
Influent and effluent wastewater samples were collected from 2 WWTPs in Arizona monthly for a year, from Aug 2011 to July 2012.

**WWTP-A**

- Screen
- Primary sedimentation
- Activated sludge
- Secondary sedimentation
- Chlorination
- Effluent

**WWTP-B**

- Screen
- Primary sedimentation
- Trickling filter
- Secondary sedimentation
- Chlorination
- Effluent
Simultaneous Recovery of Virus and Protozoa

Negatively charged filter method: simple, rapid, and inexpensive and can simultaneously recover virus, protozoa, and bacteria.

- (RT-)qPCR
- Process control
- Immunomagnetic separation (IMS)
- Microscopic count
- PCR-sequencing
- Protozoa elution
- Bacteria PCR

$3 / sample
$11 / sample
ENTEROVIRUS

- Traditional viral target, easily culturable
- Well studied RNA virus

**WWTP-A**

<table>
<thead>
<tr>
<th>Month</th>
<th>2011</th>
<th>2012</th>
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<tbody>
<tr>
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<td>6</td>
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<tr>
<td>Oct</td>
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<td>Dec</td>
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<td>Mar</td>
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<td>6</td>
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<td>Jul</td>
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<td>6</td>
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**WWTP-B**

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<td>Jun</td>
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<tr>
<td>Jul</td>
<td>8</td>
<td>7</td>
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</table>
ADENOVIRUS

- Traditional viral target, culturable
- Well studied DNA virus
- Highly persistent in the environments, high UV resistance
GI NOROVIRUS

- Noroviruses: major cause of viral gastroenteritis.
- Five genogroups: GI, GII, GIV infect humans.

**Noroviruses:**

- Major cause of viral gastroenteritis.
- Five genogroups: GI, GII, GIV infect humans.
AICHI VIRUS

- May cause gastroenteritis in humans.
- Emerging waterborne virus.

First data on the occurrence of Aichi virus in the environments in the US.
Pepper Mild Mottle Virus

- Plant virus that infects chili and bell peppers.
- Dietary origin (e.g., up to $10^7$ virus/ml in Tabasco)
- Extremely stable, pass through the human gut.

Highly abundant in influent and effluent throughout the year (up to $10^7$ copies/L)
SUMMARY

1. Novel viruses
First quantitative data on the seasonal occurrence of GIV norovirus, sapovirus, Aichi virus, and Pepper Mild Mottle virus in wastewater in the US.

2. Novel “viral tracers”
Aichi virus and Pepper Mild Mottle virus were highly abundant in wastewater: conservative viral indicators in wastewater reclamation systems.
MODEL VIRUS INDICATOR: Pepper Mild Mottle Virus

Based on:

- Abundance
- Lack of seasonality
- Not removed during treatment
CURRENT SUCCESS STORY

Akrum Tamimi, Ph.D. & Chuck Gerba, Ph.D.

- MagnaGro Process™ - Patented Process utilizes sodium metam
- Inactivates Enteric Virus, Helminth (Ascaris) Eggs and Fecal Coliform
- Meets Vector Attraction Reduction (VAR)
- Low Cost and Takes 24 hours
- 4 Years of data generated at University of Arizona
- Accepted by EPA as a “Process to Further Reduce Pathogens (PFRP) Equivalency in Biosolids i.e. A new technology for meeting Class A pathogen requirements While fulfilling Vector Attraction Reduction
- The 13th EPA Equivalency since 1987
- Can be Implemented by Magna Flow Environmental Inc.
ANTIBIOTICS AND WASTEWATER TREATMENT
ANTIBIOTICS

Natural products from cultured microbes

- Penicillin from *Penicillium notatum* (Alexander Fleming, 1929)
- Streptomycin from *Streptomyces griseus* (Selman Waksman, 1943)

**ISSUE:** the more antibiotics are used, the more resistant bacteria become
MODE OF ACTION OF ANTIBIOTICS

- Antibiotics and their targets
- Cell Wall Synthesis: Penicillin, Vancomycin
- DNA-Gyrase: Nalidixic acid
- DNA-Directed RNA-Polymerase: Rifampicin
- 50S Inhibitors: Erythromycin, Chloramphenicol
- 30S Inhibitors: Tetracyclin, Kanamycin, Streptomycin, Gentamicin
- RNA-Elongation: Actinomycin
- Structure of Cytoplasmic Membrane: Polymyxin
- Translation
- Transcription
- Protein Synthesis via tRNA: Puromycin
Does Increasing Solids Retention Time in the Wastewater Treatment Process Affect the Persistence of Antibiotic Resistance Genes?

Stefan Walston
M.S. Graduate Student
DEGRADATION OF TRACE ORGANICS (ANTIBIOTICS) DURING TREATMENT

**Question:** Does increased SRT lead to reduced levels of antibiotics?
Wastewater Treatment Plants (WWTPs)

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.
PROPOSED OUTCOME

Concentration

Solids Retention Time

TrOC

AR
METHODS

Molecular & Cultural Analysis

Chemical Analysis

Bacterial Isolates (MIC)  Quantitative PCR  SPE  LC-MS/MS
SRTs Vary Among WWTPs

<table>
<thead>
<tr>
<th>WWTP Site</th>
<th>SRT (days)</th>
<th>BOD (mg/L)</th>
<th>MGD</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>1-2</td>
<td>243</td>
<td>35</td>
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<td>Plant</td>
<td>2-4</td>
<td>253</td>
<td>9</td>
<td>CAS/Cl/UV</td>
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<tr>
<td>Plant</td>
<td>4</td>
<td>263</td>
<td>8</td>
<td>MBR</td>
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<td>Plant</td>
<td>8-9</td>
<td>167</td>
<td>9</td>
<td>CAS/Cl/UV</td>
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<tr>
<td>Plant</td>
<td>14</td>
<td>210</td>
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<td>CAS/Cl/UV</td>
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<td>17</td>
<td>245</td>
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<td>CAS/Cl/UV</td>
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<tr>
<td>Plant</td>
<td>19</td>
<td>328</td>
<td>135</td>
<td>CAS/Cl/UV</td>
</tr>
<tr>
<td>Plant</td>
<td>25</td>
<td>282</td>
<td>2</td>
<td>SBR</td>
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</tbody>
</table>
CLINICALLY RELEVANT ANTIBIOTICS

**Target Antibiotic Resistance Genes**

- sulfamethoxazole: sul(I) & sul(II)
- trimethoprim: dfr1A
- ampicillin: ampC
- tetracycline: tetW
- vancomycin: vanA
• Approximately 72 samples were collected from 8 WWTPs
• 288 pure isolates were selected from media plate
• Each isolate were screened against varying antibiotic concentrations according to clinical standards
Resistance to Vancomycin
($\geq 4 \mu g/ml$)

Increase in Antibiotic Concentration

Growth above this conc. is resistant

0 µg/ml  0.5 µg/ml  1 µg/ml  2 µg/ml  4 µg/ml  8 µg/ml  16 µg/ml  32 µg/ml
ANTIBIOTIC CONCENTRATION RANGE
(µg/ml)
Vancomycin

Increase in Antibiotic Concentration

Growth above this conc. is resistant
# RESISTANT BACTERIAL ISOLATES

<table>
<thead>
<tr>
<th>% of Resistant Isolates</th>
<th>VANCOMYCIN</th>
<th>Bacterial Isolates</th>
<th>Bacterial Isolates</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Primary</td>
<td>Secondary</td>
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<tr>
<td>SRT of 3 days</td>
<td></td>
<td>95%</td>
<td>63%</td>
</tr>
<tr>
<td>SRT of 9 days</td>
<td></td>
<td>96%</td>
<td>90%</td>
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<tr>
<td>SRT of 19 days</td>
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<td>94%</td>
<td>83%</td>
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- Clinical isolates die during treatment
- As SRT ↑ more resistance induced
MOLECULAR ANALYSIS

• Results indicate the presence of all target genes along the treatment train.
• Quantitative data indicate ARGs are decreasing along the treatment train.
• Normalized to the TOTAL number of copies of Bacterial 16S rRNA genes.
TRACE ORGANIC ANALYSIS

- Solid phase extractions are complete and archived.
- LC-MS standards are complete.
- LC-MS/MS is currently underway at the Arizona Laboratory of Emerging Contaminants.
CURRENT FINDINGS

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<tbody>
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<td>Plant</td>
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<td>Plant</td>
<td>14</td>
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<td>Plant</td>
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<td>Plant</td>
<td>19</td>
</tr>
<tr>
<td>Plant</td>
<td>25</td>
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</tbody>
</table>

Antibiotic Resistant Genes are decreasing along the treatment train

All WWTPs are effective at lowering the percentage of resistant bacterial isolates to some degree

Future investigation should include tertiary treatment and evaluate final effluent
ONGOING OBJECTIVE

![Graph showing Concentration vs. Solids Retention Time with TrOC, Critical Value, and AR points.](image)
INFLUENCE OF BIOSOLIDS ON SOIL ANTIBIOTIC RESISTANT BACTERIA
### Antibiotic-resistant bacteria in environmental samples and foods.

<table>
<thead>
<tr>
<th></th>
<th>Antibiotic Numbers CFU/gm. or ml</th>
<th>Heterotrophic Plate Count ---- CFU/G or ml ----</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample</strong></td>
<td><strong>Tetracycline</strong></td>
<td><strong>Ciprofloxacin</strong></td>
</tr>
<tr>
<td><strong>Environmental Samples</strong></td>
<td></td>
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<tr>
<td>Tap water</td>
<td>10^1</td>
<td>10^1</td>
</tr>
<tr>
<td>Irrigation well water</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Soil</td>
<td>10^5</td>
<td>10^6</td>
</tr>
<tr>
<td>Biosolids</td>
<td>10^7</td>
<td>10^7</td>
</tr>
<tr>
<td>Manure</td>
<td>10^5</td>
<td>10^6</td>
</tr>
<tr>
<td>Compost</td>
<td>10^6</td>
<td>10^6</td>
</tr>
<tr>
<td>Dust</td>
<td>10^6</td>
<td>10^6</td>
</tr>
<tr>
<td><strong>Food</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>10^5</td>
<td>10^4</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>10^1</td>
<td>10^1</td>
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<tr>
<td>Chicken</td>
<td>10^2</td>
<td>10^3</td>
</tr>
<tr>
<td>Ground beef</td>
<td>10^4</td>
<td>10^4</td>
</tr>
</tbody>
</table>
SUMMARY: Antibiotic Resistant Bacteria

- Land applied biosolids did not influence ARB concentrations
- Soil is the original source of antibiotics
- Exposures more common from handling foods
- Ready to eat lettuce—greatest relative concentrations of ARB
- Hospitals more important
- Concentrated animal feedlot operations also critical
WHAT IS WEST?

- A partnership between the University of Arizona and Pima County Wastewater to promote research and business development of:
  - Water treatment technologies
  - Contaminant minimization and production
  - Innovative education and training

- Goal is to solve challenging water and energy sustainability issues locally, nationally and internationally
NEW WEST COMPLEX

- Pima County will fund and construct a new 22,000 square foot building and lease to University of Arizona for cost of utilities


- Founders/Directors: Ian Pepper and Shane Snyder
THE NEW WEST BUILDING

- Administrative Offices, Conference Rooms, Student Study Areas
- Molecular Microbiology Lab
- Real-Time Sensor Lab
- Aquatic Toxicology Lab
- Water Treatment Lab
- Energy Lab
- Hi-Bay area for Demonstration Laboratory Water Treatment Technologies
THE NEW WEST CAMPUS

- New state-of-the-art water reclamation facility
- Adjacent to Tucson Water constructed wetlands and infiltration basins
- Pima County included “tie-ins” to full-scale plant to allow for demonstration scale technologies
- Large solar energy field in place
- Ideal location for growing algae for biofuels
- Ideal location for other solar-thermal evaluations - such as biosolid disinfection
WEST MEMBERSHIP

- Currently consists of 20 members
- Public Sector, Private Sector, Regional organizations and Universities
- All members have already given cash or equipment/technology donations to WEST
- Members without offices in Tucson are being encouraged to move staff to the region as part of the WEST mission for economic development in the region
- Members are aiding in the development of short-courses for training certificates in various methods and technologies
WEST WATER MEMBERS:

- City of Peoria
- City of Tucson Water
- County Sanitation Districts of Los Angeles
- Pima County Regional Wastewater Reclamation
- Singapore Public Utilities Bureau – pending confirm.
- KAUST Saudi Arabia – pending confirm.
- Technical University of Munich – verbally confirmed
WEST WATER MEMBERS:

Private Sector

- Alticor / Access Business Group
- AvraGro Systems, Inc.
- Heliae
- Instant BioScan
- Mannco Environmental Services
- Agilent Technologies
- Dow Chemical
- Woongjin Coway
- Xylem Water
- Trojan UV Corporation
- Hach Corporation
WEST WATER MEMBERS

• Arizona Department of Environmental Quality

• King Abdullah University of Science & Technology (KAUST)

• California Association of Sanitation Agencies

• Northwest Biosolids Management Association
WEST FUNDING

- Research support via extramural grants
- UA support (CALS & ENG)
- Industrial Advisory Board (IAB) Membership
- Income from UA summer classes
- Personnel training
- State-certified routine lab analyses
- Philanthropy from community
## WEST: Current Major Initiatives

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<tr>
<th>Agency</th>
<th>Amount</th>
<th>Status</th>
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<tbody>
<tr>
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<td>$4.1m</td>
<td>Submitted</td>
<td>Lansey, Snyder, Pepper, Gerba</td>
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<tr>
<td>NSF</td>
<td>$0.9m</td>
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<td>Pepper</td>
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<tr>
<td>AID</td>
<td>$18m</td>
<td>Pre-proposal accepted</td>
<td>Tamimi, Gerba, Pepper</td>
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<td>$4m</td>
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<td>Snyder, Pepper, Gerba, Lansey, Arnold, etc.</td>
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<tr>
<td>WRRF</td>
<td>$1.7m</td>
<td>Successful</td>
<td>Pepper &amp; Snyder</td>
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RELEVANT FUNDED PROJECTS

• Evaluation of Class A Pelletized Biosolids for the Growth of Cotton
• Evaluation of *Ascaris* Egg Viability in Biosolid Amended Soil
• Evaluation of Pathogen Removal at a New Wastewater Treatment Plant that Utilizes Dissolved Air Flotation and a 5-Stage Bardenpho Process
• Risk Assessment of *Legionella* in Reclaimed Water
WEST IMPACT: International Collaborations

- Australia
  - University of Adelaide
  - Commonwealth Scientific and Industrial Research Organization (CSIRO)
- Singapore - National University of Singapore and Singapore PUB
- Saudi Arabia – King Abdullah U. of Science & Technology and King Abdulaziz University
- Europe – Anglian Water, Suez Environment, PWN Technologies, U. of Catania, TU Munich
WEST IMPACT:  
Training Component

- Utility operators and technicians trained on advanced water treatment technologies i.e., RO, UF, UV, Ozone, others

- Technology evaluation and testing i.e., sensors, energy efficient pumps, oxidation, membranes, etc.

- Energy projects – thermo-solar, biofuels, biogas (methane/hydrogen) from digestors
"The WEST Center will target the water-energy nexus by ensuring a supply of safe drinking water to meet community needs for the foreseeable future, while meeting sustainable energy requirements."

Ian Pepper, Feb. 2013

"In addition, the WEST Center aspires to not only become a global leader in new water and energy technologies, but also focus on creating additional jobs and economic development in the region, while simultaneously providing advanced educational and training opportunities."

Shane Snyder, Feb. 2013