Antibiotic Resistant Bacteria and Genes in Primary to Tertiary Treated Wastewater

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Background

• “Superbugs”, multi-antibiotic resistant bacteria, have been recognized as an emerging public health threat

• Historically, the focus has been on hospital-acquired infections

• Attention has shifted to include concern over antibiotic resistant bacteria released into the environment
Deadly superbugs from hospitals get stronger in the sewers and could end up in the Pacific Ocean.

‘Nightmare bacteria’ resistant to antibiotic of last resort found in U.S.

Superbug known as ‘phantom menace’ on the rise in U.S.

Antibiotic resistance: The grim prospect
Wastewater treatment may enhance levels of Antibiotic Resistant Bacteria

• Processes such as aerobic digestion encourage bacterial growth and may serve as an incubator for antibiotic resistant bacteria

• Residence time and rapid growth during treatment may promote transfer of antibiotic resistance genes

• Little work has been done to quantify the effect of treatment on levels of antibiotic resistant bacteria in wastewater
Study Questions

• Do wastewater treatment facilities in Southern California discharge antibiotic resistant bacteria or genes?
  • If so, what kind?

• Are the number of antibiotic resistant bacteria or genes in wastewater discharges higher or lower than in the input?

• How does level of treatment affect levels of antibiotic resistant bacteria or genes?
Approach

• Paired influent and final effluent samples to compare levels of antibiotic resistant bacteria before and after treatment

• Multiple plants to capture across-plant differences in treatment regimens

• Four sampling events (once per quarter) to capture seasonal variability

• Genetic methods to identify and quantify resistance genes
<table>
<thead>
<tr>
<th>POTW</th>
<th>Final Effluent Treatment</th>
<th>Agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperion Water Reclamation Plant</td>
<td>Advanced Secondary Treatment (No Disinfection)</td>
<td>City of Los Angeles</td>
</tr>
<tr>
<td>Terminal Island Water Reclamation Plant</td>
<td>Tertiary Treatment</td>
<td>City of Los Angeles</td>
</tr>
<tr>
<td>Joint Water Pollution Control Plant</td>
<td>Advanced Secondary Treatment (Disinfection)</td>
<td>Los Angeles County Sanitation District</td>
</tr>
<tr>
<td>Water Reclamation Plant</td>
<td>Tertiary Treatment</td>
<td>Los Angeles County Sanitation District</td>
</tr>
<tr>
<td>Plant No. 1</td>
<td>Advanced Secondary Treatment</td>
<td>Orange County Sanitation District</td>
</tr>
<tr>
<td>Plant No. 2</td>
<td>Advanced Secondary Treatment</td>
<td>Orange County Sanitation District</td>
</tr>
<tr>
<td>North City Water Reclamation Plant</td>
<td>Tertiary Treatment</td>
<td>City of San Diego</td>
</tr>
<tr>
<td>Pt. Loma Wastewater Treatment Plant</td>
<td>Advanced Primary Treatment</td>
<td>City of San Diego</td>
</tr>
<tr>
<td>South Bay Water Reclamation Plant</td>
<td>Secondary Treatment</td>
<td>City of San Diego</td>
</tr>
<tr>
<td>South Bay International Wastewater Treatment Plant</td>
<td>Secondary Treatment</td>
<td>City of San Diego/IBWC</td>
</tr>
</tbody>
</table>
# High Priority Antibiotic Resistant Bacteria

<table>
<thead>
<tr>
<th>Antibiotic Resistant Bacteria</th>
<th>Bacterial Groups</th>
<th>Selective Media</th>
<th>Antibiotic Family</th>
<th>Motivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenem Resistant Enterobacteriaceae (CRE)</td>
<td>Fecal coliforms, Klebsiella</td>
<td>mFC; mod mFC</td>
<td>Beta-lactam</td>
<td>EPA study found CRE in wastewater; last-line antibiotic</td>
</tr>
<tr>
<td>Vancomycin Resistant Enterococci (VRE)</td>
<td>Enterococcus spp</td>
<td>mEI</td>
<td>Glycopeptide</td>
<td>Sewage spills at FL beaches found persistent VRE; last-line antibiotic for MRSA</td>
</tr>
<tr>
<td>Methicillin Resistant Staphylococcus aureus (MRSA)</td>
<td>Staphylococcus aureus; Staphylococcus spp.</td>
<td>CHROMAgar SA</td>
<td>Beta-lactam</td>
<td>Studies have found it in California ambient water</td>
</tr>
</tbody>
</table>
Quantification Methods: Viable ARB

- Quantification and isolation of high priority antibiotic resistant bacteria by culture
  - CRE-FC, CRE-Kleb, VRE, MRSA
# Antibiotic Resistance Genes

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Antibiotic Resistance Gene</th>
<th>Gene Names</th>
<th>Method</th>
<th>Sample Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted</td>
<td>carbapenemase</td>
<td>KPC, OXA</td>
<td>Quantitative or Digital PCR</td>
<td>Isolates; Wastewater samples</td>
<td>Precise detection and quantification of a specific gene</td>
</tr>
<tr>
<td>Screening panel</td>
<td>List of ARGs</td>
<td>Multiple</td>
<td>Quantitative PCR</td>
<td>Isolates; Wastewater samples</td>
<td>Detection and quantification of multiple genes</td>
</tr>
<tr>
<td>Survey</td>
<td>Resistome</td>
<td>Multiple</td>
<td>Sequencing</td>
<td>Wastewater samples</td>
<td>Detection of ARGs in a sample</td>
</tr>
</tbody>
</table>
Results: Antibiotic Resistant Bacteria

Are there antibiotic resistant bacteria in discharge?
Yes
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Is the number higher or lower than in the influent?
Lower (mostly)
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Are there antibiotic resistant bacteria in discharge?
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Lower (mostly)

What is the effect of treatment?
As treatment increases, ARB numbers decrease
Percent ARB by High Priority Group

- **CRE-FC:** Primary
- **CRE-Kleb:** Secondary
- **VRE:** Tertiary
- **MRSA:** Primary
Results: Antibiotic Resistance Genes

Are there antibiotic resistance genes in discharge?
Yes

Is the number of genes higher or lower than in the influent?
Lower

What is the effect of treatment?
As treatment increases, ARGs decrease
Percent Carbapenemase Genes in Effluent

- Primary: 60%
- Secondary: 10%
- Tertiary: 0%
The Good, The Bad, The Unknown

• Higher treatment levels reduce the number of ARB and ARG in effluent

• ARB and ARG are being discharged

• What happens to the ARB and the ARG?
  • In environmental waters?
  • Health risk?
Effluent ARB concentrations: What do these concentrations mean?

![Graph showing effluent ARB concentrations for different ARB groups (CRE-FC, CRE-Kleb, VRE, MRSA) across primary, secondary, and tertiary stages. The graph illustrates the log CFU per 100ml Effluent.]
Next Steps

• Determine extent of ARB/ARGs influence in environmental waters
  • Investigate waters around an ocean and an inland outfall
  • Compare concentrations and measure extent of plume

• Measure risk of colonization and human illness in recreational waters
  • Epidemiology studies: expensive and time-consuming
  • Study team in place and pursuing funding