Environmental Microbiology Laboratory

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Hristova Research Program

- Environmental and Human Health
- Antibiotics, Antibacterials, Antibiotic Resistance
- Nanomaterials, Nanocomposite polymers
- Manure or Organic Pollutants (Oil, PAHs)
- MMSD funding
- NOAA WI Sea Grant
- NIEHS-Children's' Center
- NSF U/ICRC
- Marquette Innovation
- AnGbioGcs, AnGbacterials AnGbioGc Resistance Nanomaterials Nanocomposite polymers
- NSF, U/ICRC
Surface and Groundwater Contamination in Kewaunee County, WI

- WI has the largest number of dairy farms in the United States
- KC is home to 16 Concentrated Animal Feeding Operations (CAFOs); 4 Wastewater Treatment Plants (WWTPs)
- There are 98,000 cows and 20,000 people in 1100 square miles of land
- Impaired 3 Rivers
- Unsafe drinking water wells
- Point and Non-point source of pollution
The water cycle

- **Driving Forces**
  - Solar energy
  - Gravity

- **Processes**
  - Evaporation
  - Transpiration
  - Precipitation
  - Infiltration
  - Groundwater flow
  - Overland flow
  - Stream runoff
Surface and Groundwater Contamination in Kewaunee County – Manure runoff

Sampling surface water – Sept 14th

After rain event – Sep 26th, 2016
High Nitrate Level in Kewaunee River

$\text{NO}_3^- + \text{NO}_2^-$

10 mg/ml $\text{NO}_3^-$ is the EPA standard for drinking water
High Levels of Fecal Contamination Found in Surface Water

The EPA standard for impaired river is 126 cells/100 ml for E. coli.
Water Contamination is linked to human health

• High nitrate level in drinking water sources
  - Blue- baby syndrome, reproductive defects, nervous system impacts, hyperthyroidism, insulin-dependent diabetes, etc. (Burkholder et al., 2007)
• High *E. coli* level – gastrointestinal infections
• *Staphylococcus aureus* MRSA - skin and soft tissue infections, sepsis, osteomyelitis and pneumonia
  - At first it was associated with hospitals
  - Now infections within the community are becoming more and more common
  - Livestock-associated MRSA
Water Contamination is linked to human health

- Drinking Water Systems, Hydrology, and Childhood Gastrointestinal Illness in Central and Northern Wisconsin (Mark Borchard, USDA)
  - Untreated water increases the risk of gastrointestinal illnesses in children
  - Hydrologic events also transfer pathogens into groundwater or drinking water system

- Aquatic Ecosystems as Reservoirs of Antibiotic Resistance -- Anthropogenic activities contribute to the spread of antibiotic resistance in the environment
  - Marti, E. 2014, *Trends in Microbiology*
  - Kappell and Hristova, 2015, *Frontiers in Microbiology*
  - Majority of bacteria in aquatic ecosystems are organized in biofilms
  - This allows for exchange of antibiotic resistance genes due to close proximity of bacteria
Antibiotic Use In Livestock

- Antibiotics are often included into cattle feed in order to promote growth and to prevent/treat illness
  - Agriculture accounts for 80% of antibiotic sales
- 13.1 million kilograms of antibiotics were used on livestock in 2009
- “[Only] 10% [of livestock antibiotic sales was] used to treat active infections while the remaining nearly 90% is used for growth promotion and prophylactic care
Emergence of multidrug-resistant bacteria

• The widespread use of antibiotics has led to the emergence of multidrug-resistant strains including human pathogens
Why is AB resistance a problem?

Estimated minimum number of illnesses and deaths caused by antibiotic resistance*:

At least 2,049,442 illnesses, 23,000 deaths

*bacteria and fungus included in this report
Multidrug Resistant Bacteria are abundant in Kewaunee County

• Several bacterial strains have been isolated from sediment samples from the 3 rivers in Kewaunee County (Kewaunee, Ahnappee, East Twin River) and determined to be multidrug resistant by students from Marquette University, WI
Students in the Experimental Microbiology Lab
How resistance has been determined?

**Kirby - Bauer Method**

- Disk diffusion antibiotic sensitivity testing
  - Use AB disks to test if bacterial growth is affected by antibiotics
- Disk contain antibiotic
  - Placed on agar plate with inoculated bacteria
- Incubation (37 °C)
- Zone of inhibition measured
- Resistance determined according to the Clinical Standards Institute
Bacterial Diversity Based on Culturing from river sediment

- *Aeromonas* and *Pseudomonas* are most common multi-drug resistant bacteria
- Other Bacteria are present and are also multi-drug resistant
Multi-drug resistant *Aeromonas* and *Pseudomonas* spp. in East Twin River – Kewaunee County

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>AB from Disk Diffusion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>AMP</td>
</tr>
<tr>
<td><strong>Aeromonas</strong></td>
<td>salmonicida</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>veronii</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>allosaccharophila</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>hydrophila</td>
<td>Red</td>
</tr>
<tr>
<td><strong>Pseudomonas</strong></td>
<td>protegens</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>umsongensis</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>prosekii</td>
<td>Red</td>
</tr>
</tbody>
</table>

Legend:
- **Susceptible**
- **Intermediate**
- **Resistant**
Bacteria resistant to 3 or more antibiotics are multidrug resistant from River Water in Kewaunee Region
How are people exposed to AB resistant bacteria?
What we could do to mitigate the problem of antibiotic resistance on a global scale?

- Stop the use of antibiotics as feed at farm animals
- Do not dispose unused medications
- Develop alternatives to treat multidrug-resistant bacteria – phage therapy
What we could do to mitigate the problem of water pollution on a local scale?

• Develop technologies to provide clean water to the residents of Kewanee County
  – project with Stonehouse Technologies Inc. (GWC) funded by Marquette Innovation

• Work on changing environmental policy
  - Task force on alternative strategies for manure management (with WDNR)
  - Collaboration with Jill Birren (College of Education) working with the community