Environmental Microbiology Laboratory

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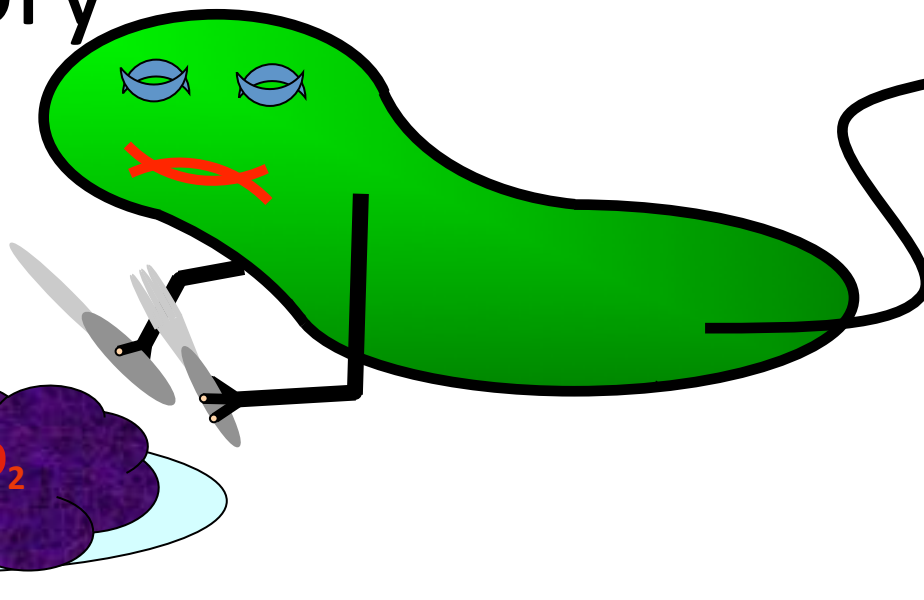
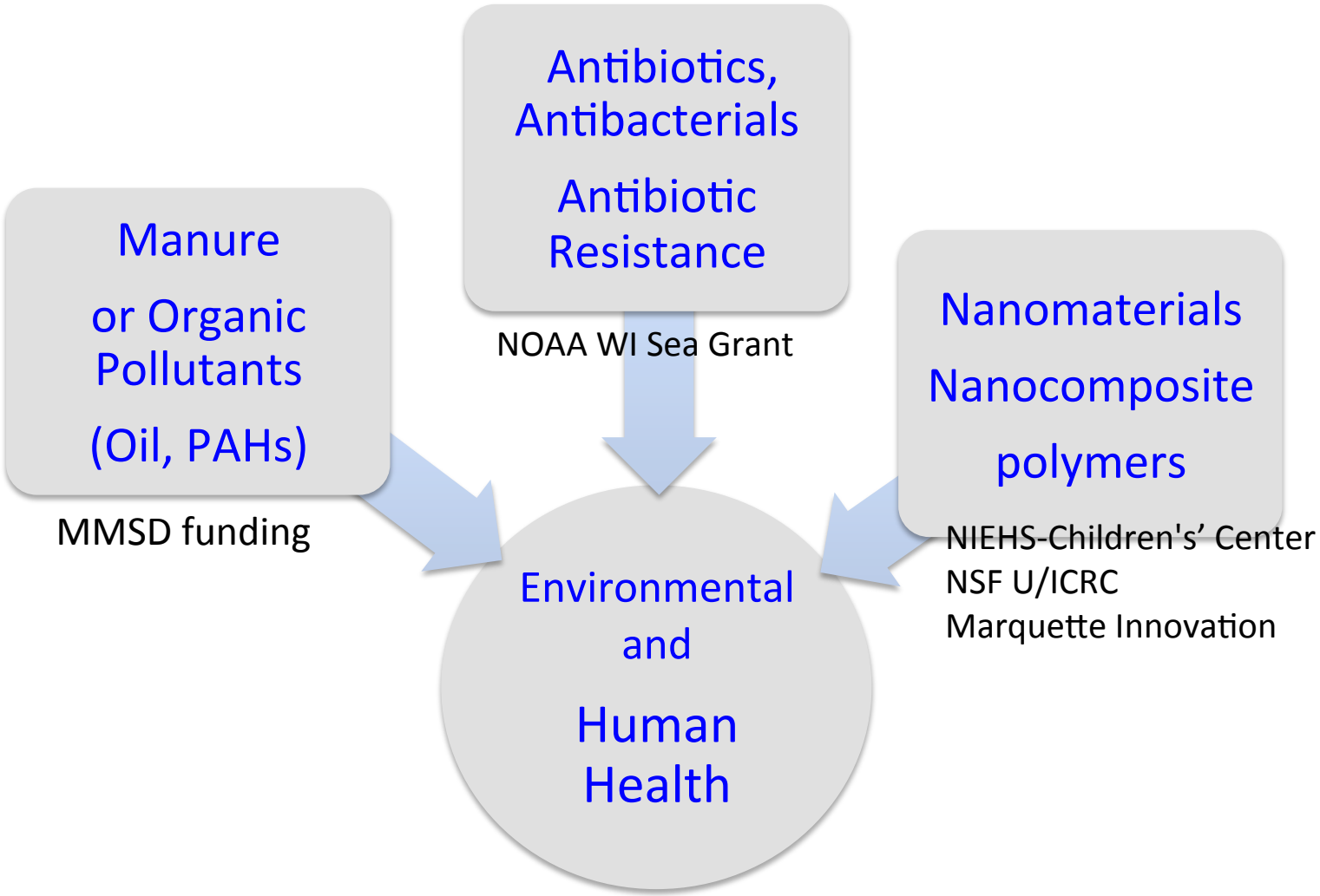
Biological Sciences

Marquette University

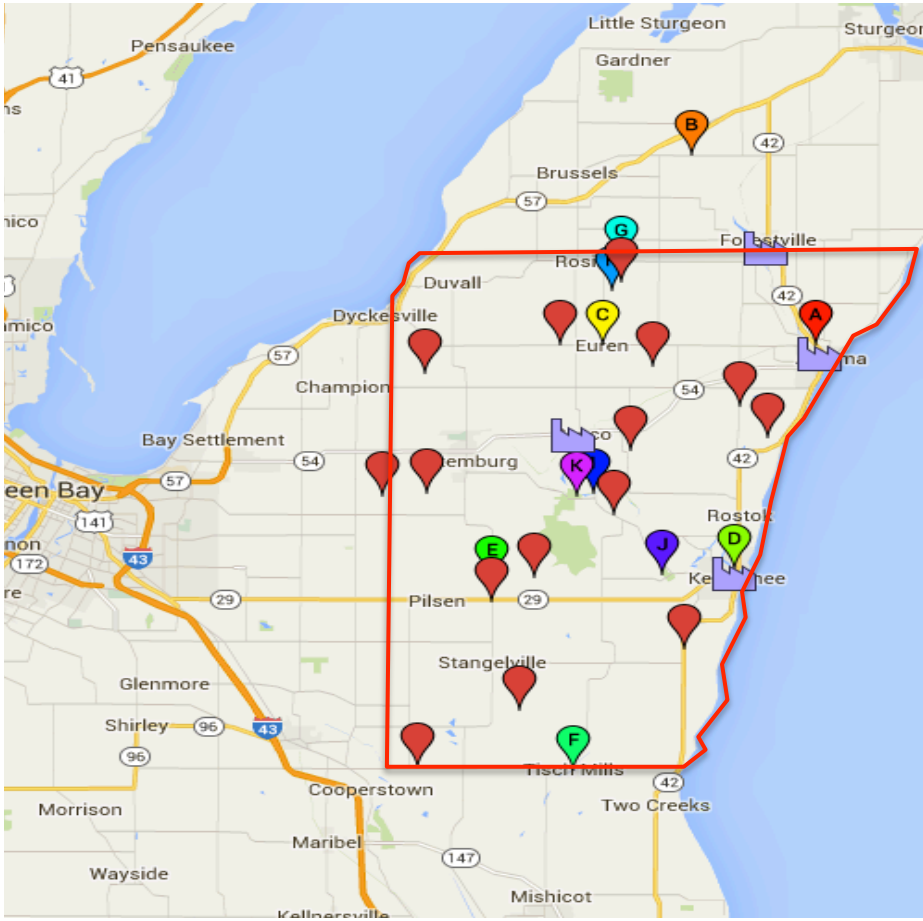


Hristova Research Program



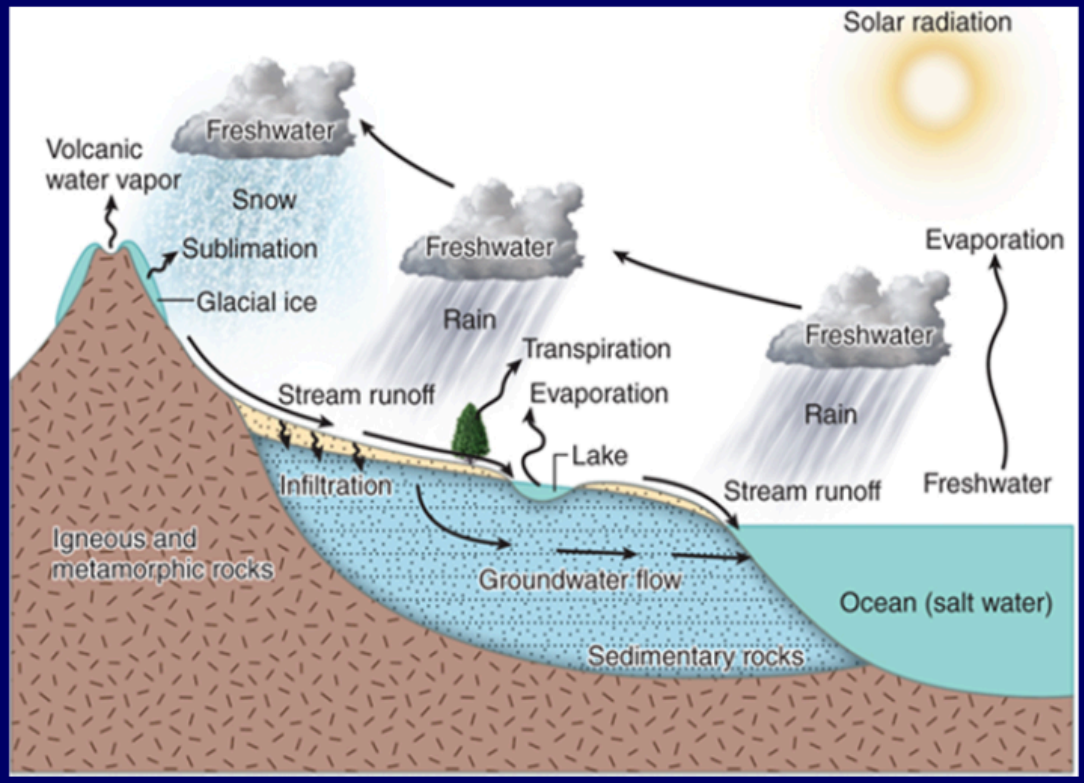
Surface and Groundwater Contamination in Kewaunee County, WI

- 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

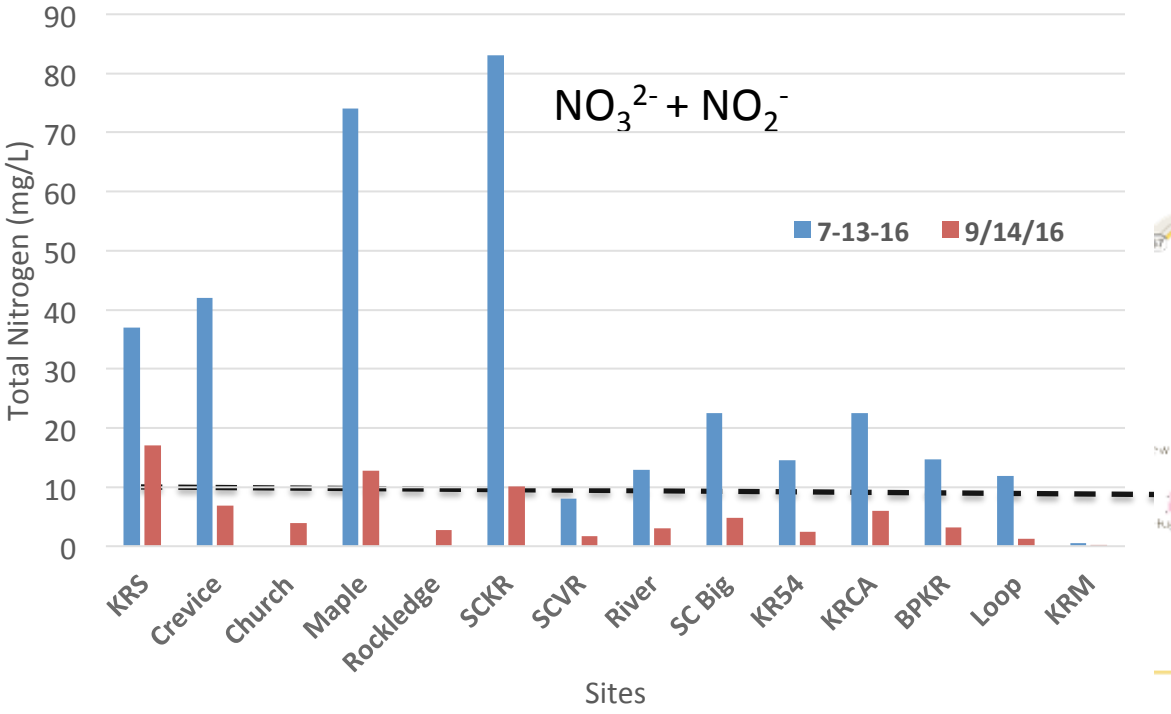


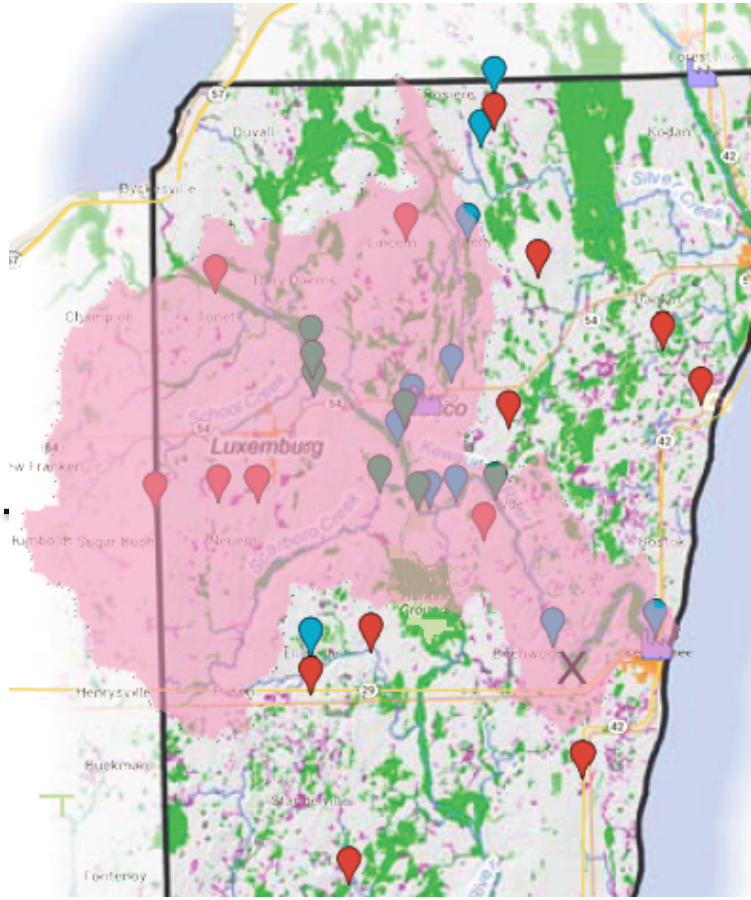
Surface and Groundwater Contamination in Kewaunee County – Manure runoff

Sampling surface water –Sept 14th

After rain event – Sep 26th, 2016

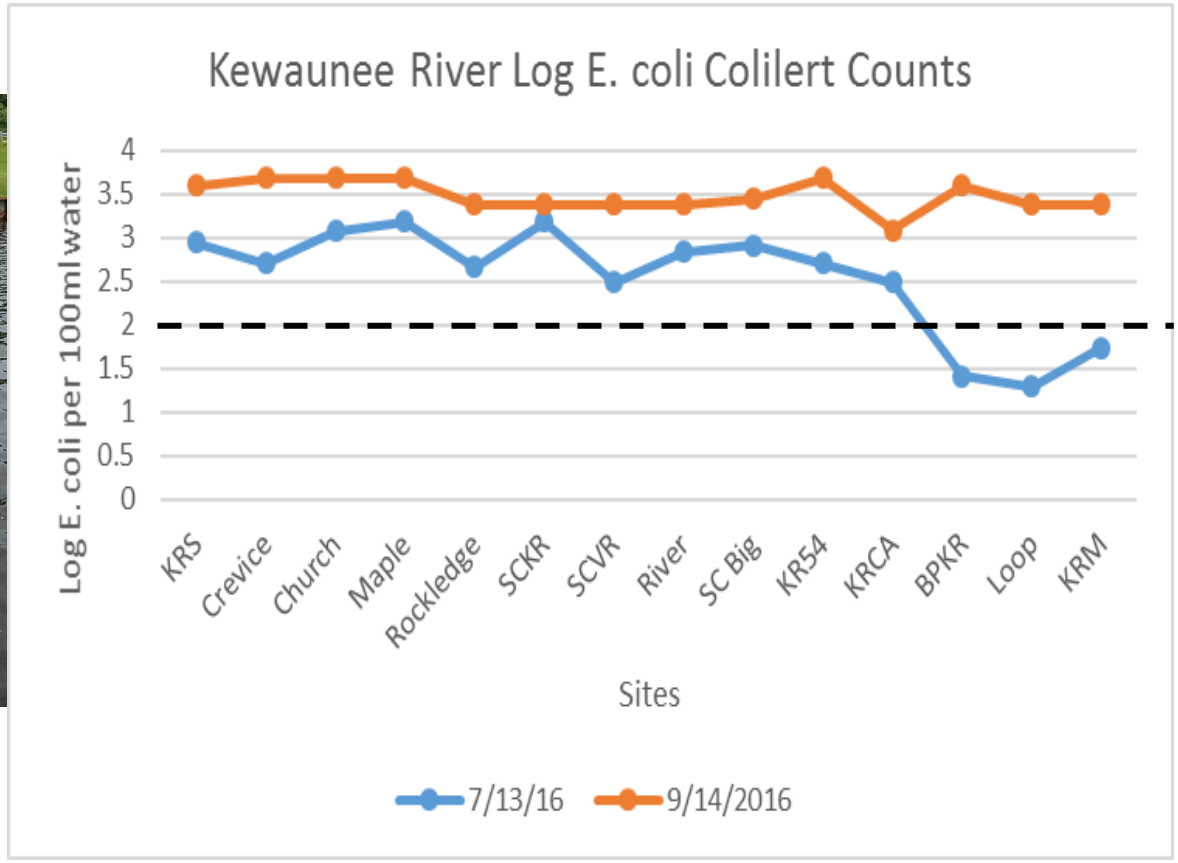
High Nitrate Level in Kewaunee River





10 mg/ml NO_3^{2-} is the EPA standard for drinking water

High Levels of Fecal Contamination Found in Surface Water



EPA standard for impaired river 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

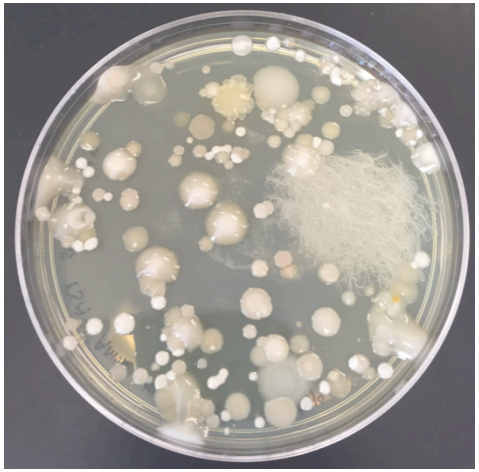
Emergence of multidrug-resistant bacteria

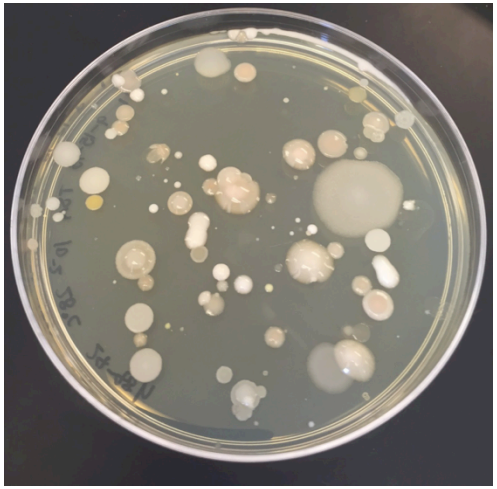
- The widespread use of antibiotics has led to the emergence of multidrug-resistant strains including human pathogens

Why is AB resistance a problem?

Multidrug Resistant Bacteria are abundant in Kewaunee County

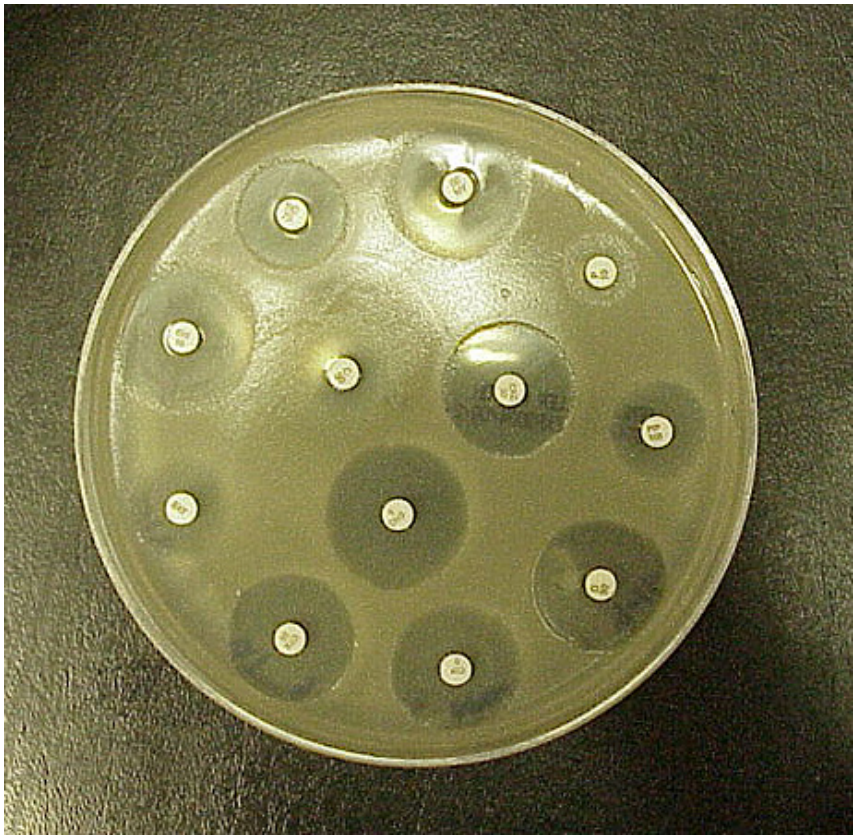
- Several bacterial strains have been isolated from sediment samples from the 3 rivers in Kewaunee County (Kewaunee, Ahnapee, East Twin River) and determined to be multidrug resistant by students from Marquette University, WI





How resistance has been determined?

Kirby - Bauer Method



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

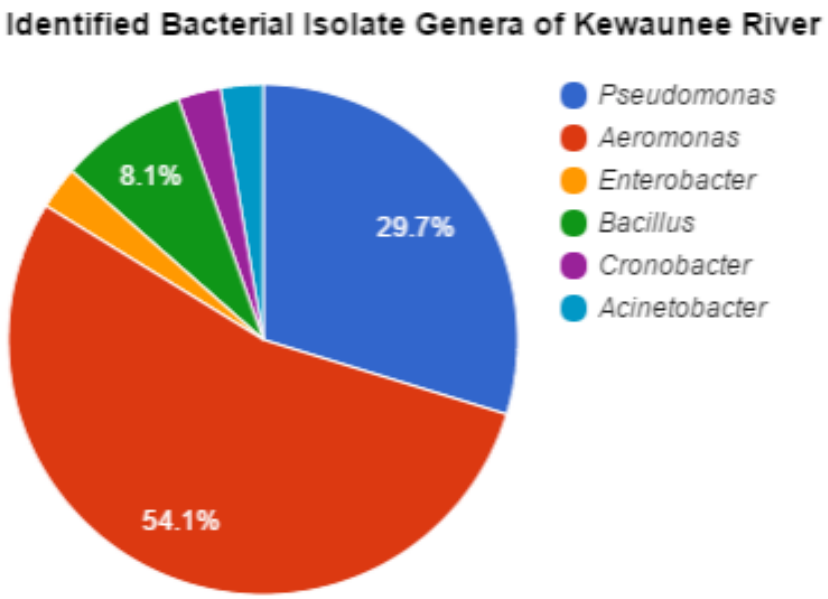
Bacterial Diversity Based on Culturing from river sediment

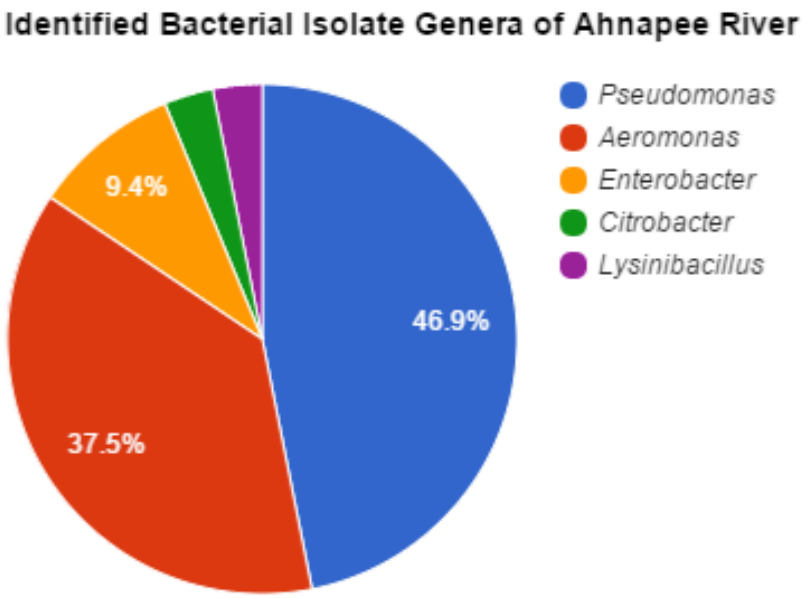
- *Aeromonas and Pseudomonas*

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

Genus	Species	AB from Disk Diffusion						
		AMP	AMOX	NEO	STREP	ERYTH	KAN	TET
Aeromonas	salmonicida							
	veronii							
	allosaccharophila							
	hydrophila							

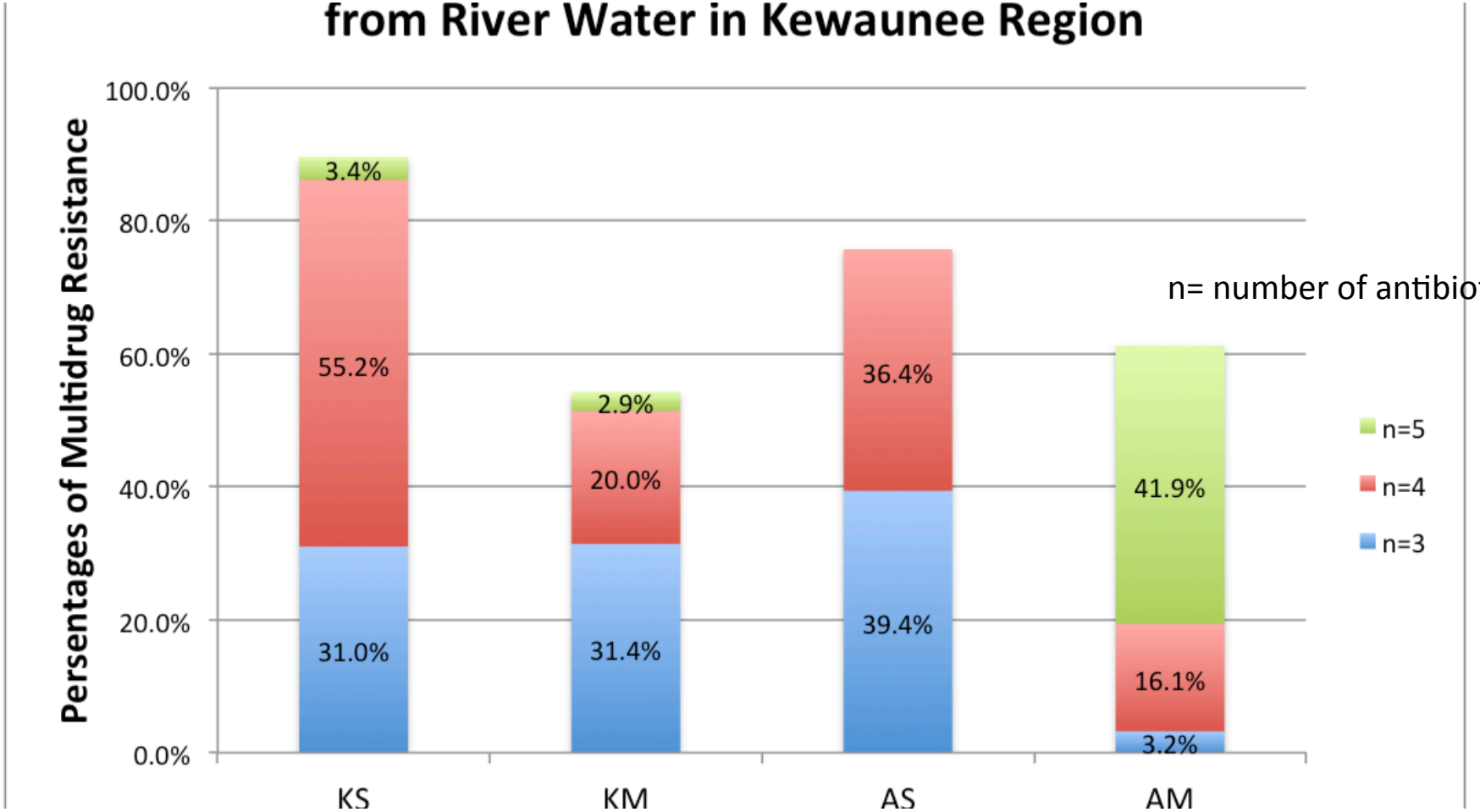
Genus	Species	AB from Disk Diffusion						
		AMP	AMOX	NEO	STREP	ERYTH	KAN	TET
Pseudomonas	protegens							
	umsongensis							
	prosekii							

Susceptible

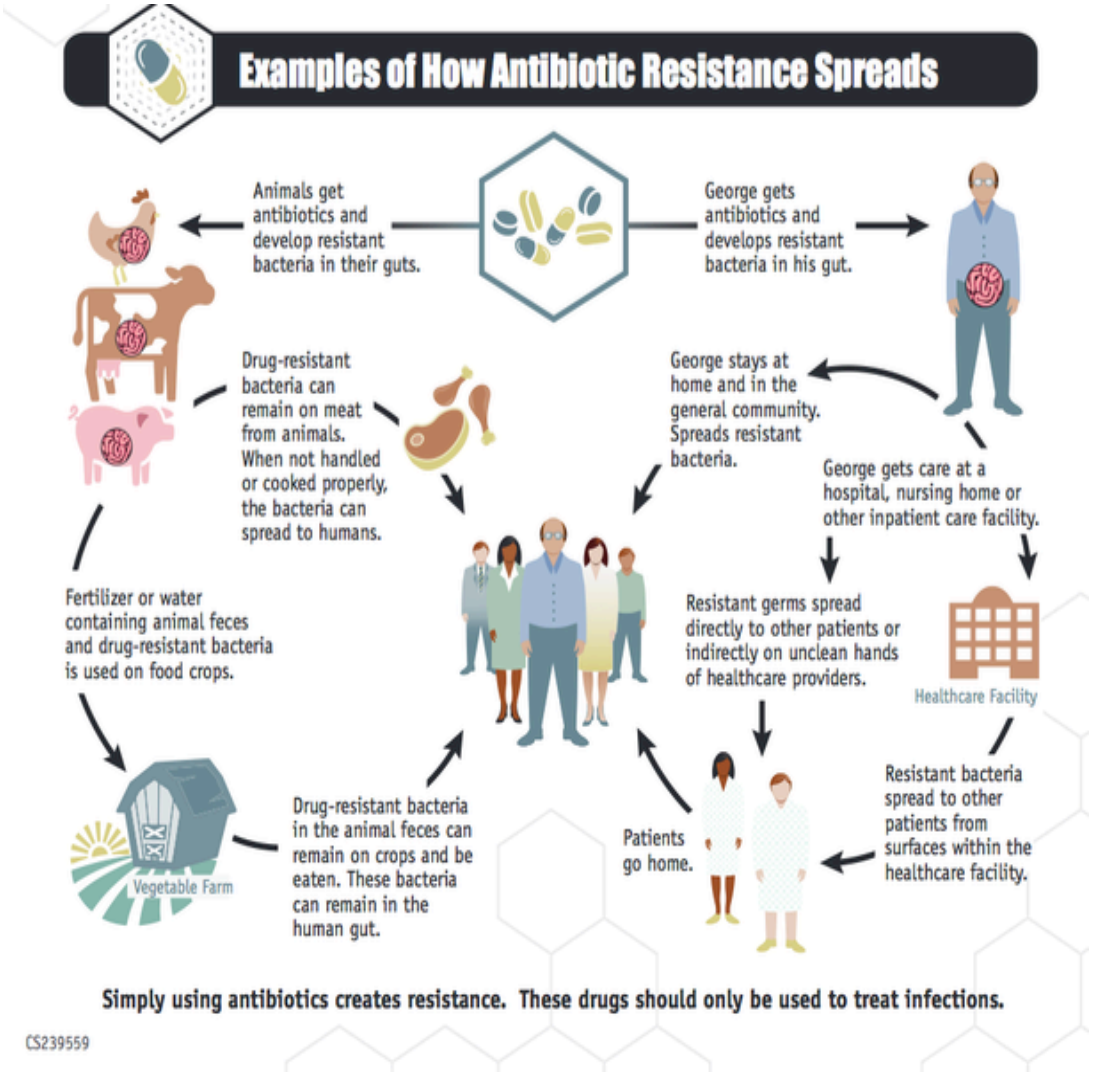
Intermediate

Resistant

Bacteria resistant to 3 or more antibiotics are multidrug resistant



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