USGS Perspective on Emerging Environmental and Public Health Topics for Michigan Waters

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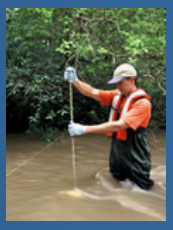
Great Lakes RESTORATION

U.S. Department of the Interior
U.S. Geological Survey

USGS science for a changing world

What is the USGS?

- A Federal science organization
- No regulatory role
- Mission is to collect and disseminate information on our natural resources, ecosystems, environment, hazards, and threats.



WWW.USGS.GOV

USGS in Michigan

Conduct studies on a variety of environmental issues

A mix of Federal programs, and studies conducted cooperatively with state, counties, municipalities

- Cooperative study design to address an issue
- Cooperative funding

Some Environmental Health Topics at the USGS

Avian Influenza, West Nile Virus (National Wildlife Health Center, Madison, WI)

Water Quality (drinking, swimming, groundwater)

Pesticides, Urban contaminants (occurrence, distribution, effects on wildlife)

Geologic Contaminants (arsenic, metals, radionuclides)

Dusts (intercontinental, soil types)

Climate Change (famine, drought, flood prediction)

Overview of Presentation

- Bacterial Pathogens in Michigan Waters
- Antibiotics and Pharmaceuticals in Michigan Waters
- Bacterial Antibiotic Resistance in the Michigan Environment

BACTERIAL PATHOGENS IN MICHIGAN WATERS

Twelve Great Lakes Beaches

Approach

- Range of land uses and settings
- Sanitary survey data recorded daily – various environmental conditions
- Samples were composites of usual number of *E. coli* samples
  - 363 samples: 277 beach water, 10 replicates, 10 field blanks, and 56 tributary or other source samples

Samples processed day after collection

- Dispersed to appropriate media
- Also filters for subsequent qPCR
- Growth frozen for DNA extraction and PCR

Bacterial Pathogens Tested

CDC reports four major pathogens associated with outbreaks of illness acquired from ambient recreational water:

- *E. coli* O157:H7 bacteria
- *Shigella spp.* bacteria
- *Cryptosporidium* spp. protozoa
- Norovirus

Salmonella and *Campylobacter* bacteria together accounted for fewer than 5% of cases, but in up to 15% of cases, the causative agent was not determined

Methicillin-resistant *Staphylococcus aureus* recently reported from marine beaches

- Source is human or animal skin—not fecal

Summary of Assays

Shiga-toxin producing *E. coli (STEC)* genes tested: *eae*A, *stx*2, *stx1*, *rfb*O157

Shigella gene tested:

- *ipa*H (specifically pathogenic *Shigella*)

Salmonella genes tested:

- *inv*A (>95% of *Salmonella*), *spv*C (pathogen *specific)*

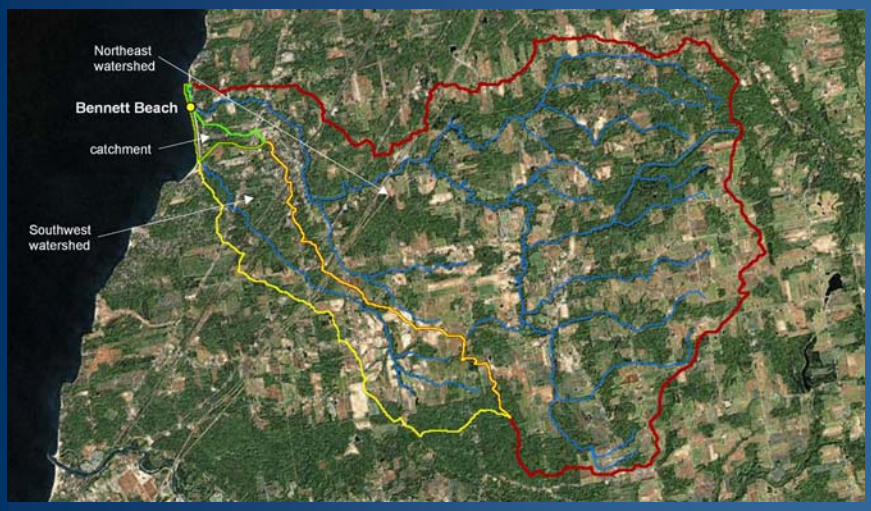
Campylobacter genes tested

- 16S rDNA for *C. jejuni* and *coli*

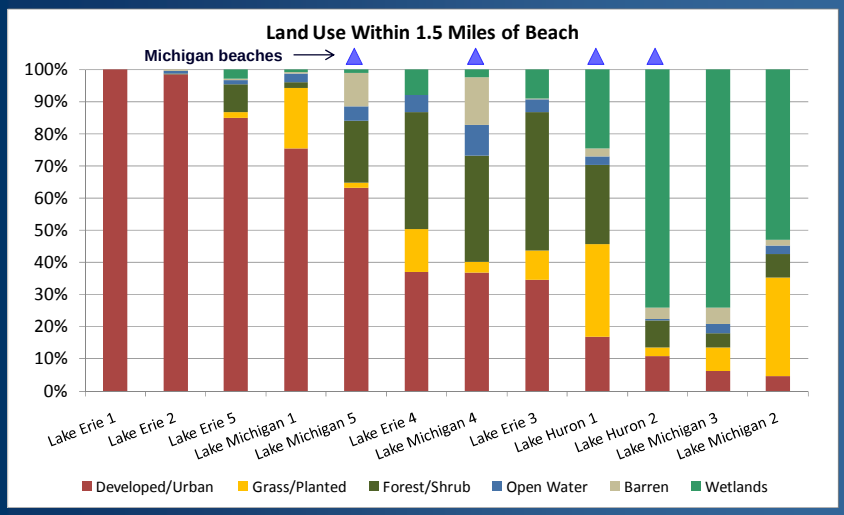
All assays performed on cultures

- Indicates viable organisms in the 100 mL beach-water sample

Land Use Defined for Multiple Source Areas



Beaches Varied By Land Use



Beach Data

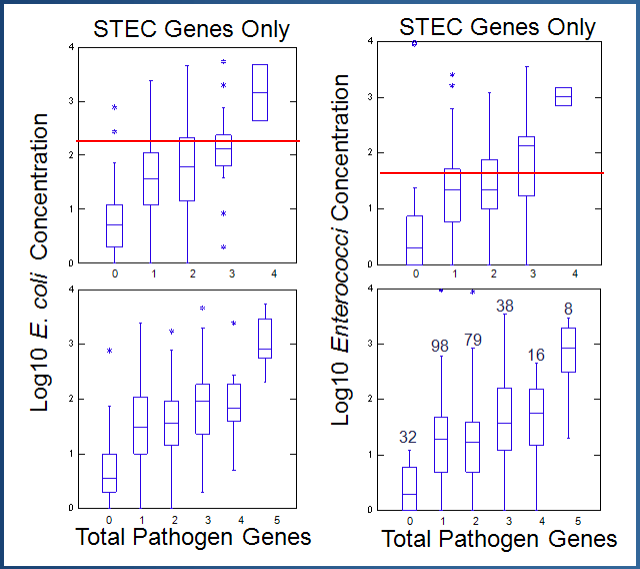
	Exceeds		FREQUENCY OF OCCURRENCE (%)												
			Shiga-Toxin producing *E. coli*						*Shigella*	*Salmonella*		MRSA			*Campylobacter*
Beach	EC	ENT	*eaeA*	*stx2*	*stx1*	*O157*	Three STEC genes	Four STEC genes	*ipaH*	*invA*	*spvC*	*mecA*	*femA*	Both MRSA genes	*jejuni* or *coli*
Lake Huron 2	0	0	43	0	10	10	5	0	0	5	0	38	0	0	5
Lake Huron 1	4	15	95	38	33	24	24	5	0	24	52	38	5	0	14
Lake Michigan 5	0	0	74	7	19	7	0	0	4	0	7	33	19	15	0
Lake Michigan 4	0	2	70	7	7	7	4	0	0	0	11	44	4	4	4

- ✓ Pathogen genes are not distributed evenly across beaches
- ✓ Beaches with high percentages of a given gene do not necessarily have high percentages of other genes
- ✓ Good water quality (no *E. coli* or enterococci exceedances) does not assure absence of pathogens

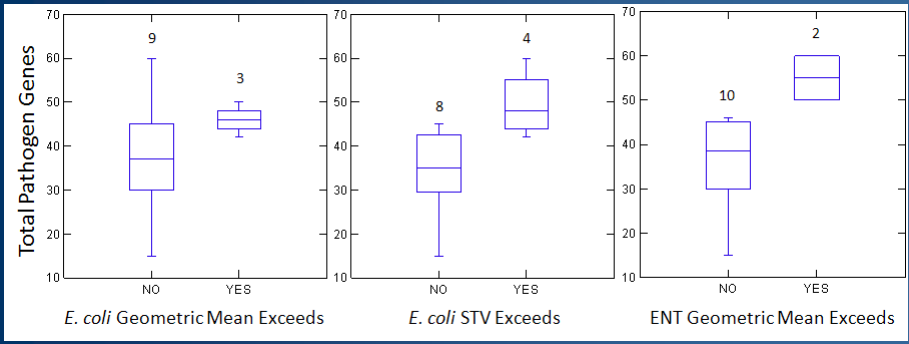
FIB Relation to Pathogen Genes in 277 Single Samples

Numerous detections in samples that meet the Single Sample Maximum

Trend toward more pathogen genes in samples that dramatically exceed the Single Sample Maximum



Beaches With Persistently High FIB Had More Pathogen Genes



STV = Statistical Threshold Level (≥ 25% of samples exceeded the *E. coli* Single Sample Maximum).

Microbial Source Tracking

Selected samples were analyzed for *Bacteroides* genes by the USGS Ohio Water Science Center

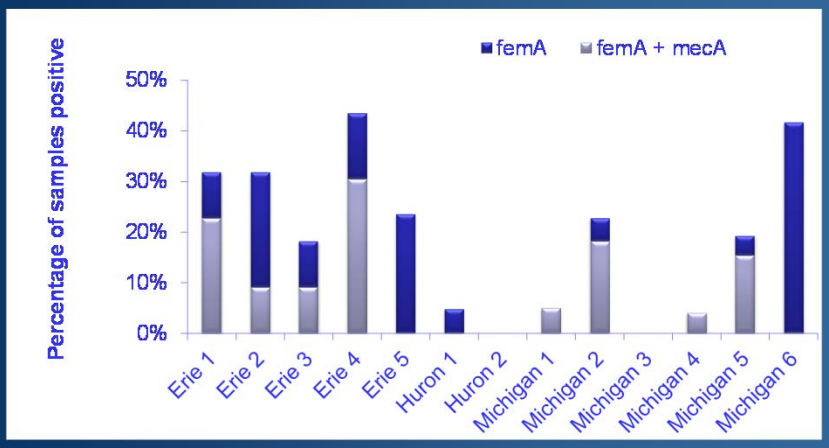
Beach	Frequency of *Bacteroides* Occurrence (%)		
	Human	Ruminant	Gull
Lake Huron 2	10	30	17
Lake Huron 1	20	10	11
Lake Michigan 5	0	0	50
Lake Michigan 4	8	23	40

Sanitary Survey Variables and Land Use May Be Predictive of Some Genes

At Michigan beaches, measures of rainfall, wave height, and wave direction were often correlated with *E. coli*, enterococci or the total number of pathogen genes

- However, *E. coli* or enterococci may be correlated with <u>different</u> variables than pathogen genes
- Suggests different processes delivering *E. coli* or pathogens to beaches
- Explains why *E. coli* may not predict pathogens

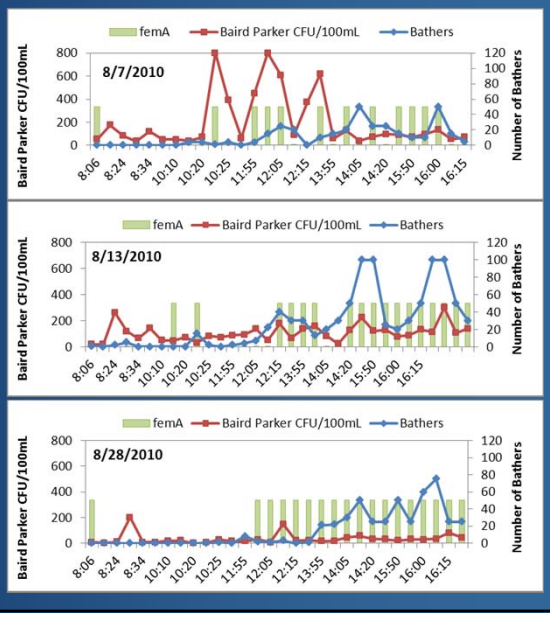
S. aureus and MRSA Potential at Beaches

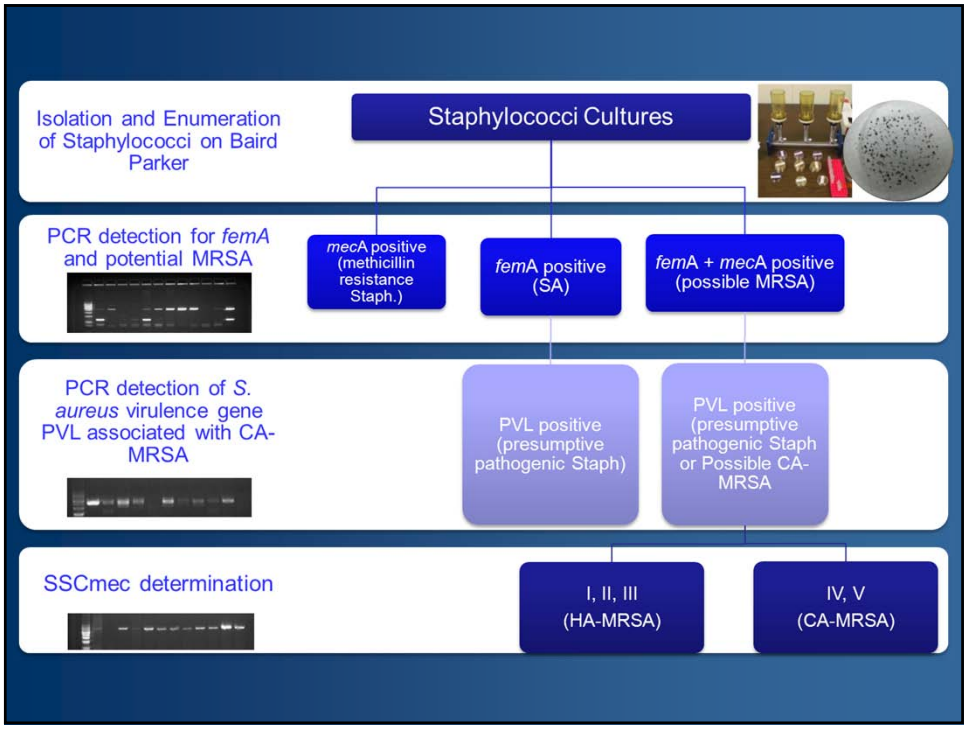


Intensive Single Beach Study

- ***fem*A and Baird Parker medium (*Staphylococcus)* counts increased in the afternoon in concert with swimmer number**
- **Suggests swimmers are the source, as indicated by the literature**
- **Early morning sampling would not detect**

Study conducted at Lake Michigan 5 beach





MRSA Isolates Were Obtained

- ✓ **Baird Parker positive black colonies**
- ✓ **CHROMagar MRSA media - positive mauve colony**
- ✓ **Mannitol salts agar + oxacillin - positive yellow colony**
- ✓ ***femA* and *mecA* positive**
- ✓ **16S rDNA sequencing resulted in 99% match to *Staphylococcus aureus* strains**
- ✓ **All (n=4) were positive for PVL**
- ✓ **3 out of 4 positive for SCCmec IV**
- ✓ **Being evaluated by Dr. Marcos Zervos at Henry Ford Hospital**

Baird Parker

Photo from www. solabia.fr

CHROMagar MRSA

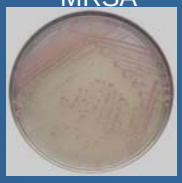


Photo from www.bd.com

Mannitol salts agar

Photo from www. fishersci.com

Example photos of MRSA on different media- Not actual isolates

Other Bacterial Pathogen Studies

- We have just completed a year-long study of multiple samplings of the St. Joseph, Grand, Au Sable, Clinton, Rouge, and Raisin rivers, during different flow conditions, testing the same genes as for the beaches study
- We have detected the genes for Shiga-toxin producing *E. coli* (STEC, including the O157 serotype) in river water within Berrien, Huron, Lenawee, and Oakland counties
- We have studied the effects of manure application on STEC transport to tile drains and streams in Lenawee County

ANTIBIOTIC-RESISTANT BACTERIA IN MICHIGAN WATERS

Why Would There Be Antibiotic-Resistant Bacteria in Water?

They are delivered with source materials that contained antibiotics, or other compounds that promoted resistance

- Sewage, septic wastes, manure, pharmaceutical processing wastes, industrial wastes containing detergents, heavy metals, other compounds

There are chemicals in the water or in the sediments that promote resistance

- Residual from prior waste

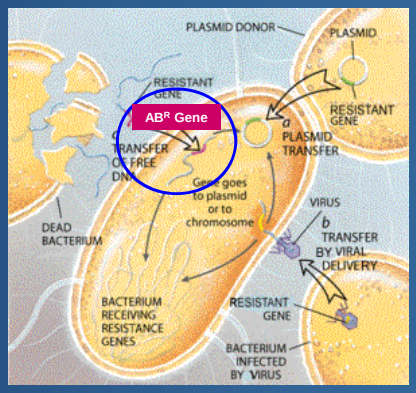
Antibiotic Resistance Gene Transfer

Antibiotic resistance (AB^R) genes are often carried on small pieces of DNA that carry other resistance genes

- Other classes of antibiotics
- Heavy metals
- Detergents
- Other organics (alcohols, phenol)

The pieces of DNA can be transferred among bacteria

Example: If a bacterium "needs" to be resistant to copper, it will keep the piece of DNA with a copper resistance gene, bringing along any AB^R genes



Modified Image from Levy SB. The Challenge of Antibiotic Resistance. *Scientific American.* March, 1998. http://www.sciam.com/1998/0398issue/0398levy.html

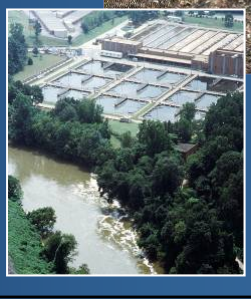
ANTIBIOTICS, PHARMACEUTICALS, AND WASTEWATER CHEMICALS IN THE MICHIGAN ENVIRONMENT

Human Sources

- WWTPs
- CSOs
- Land application of municipal biosolids or septage
- Illicit connections to storm or agricultural drains

A Michigan beach with a storm drain

MDEQ: image of a septage application violation



Agricultural Sources

Manure contains veterinary pharmaceuticals and cleaning agents, as well as pathogens

Biosolids and septage are applied to agricultural land

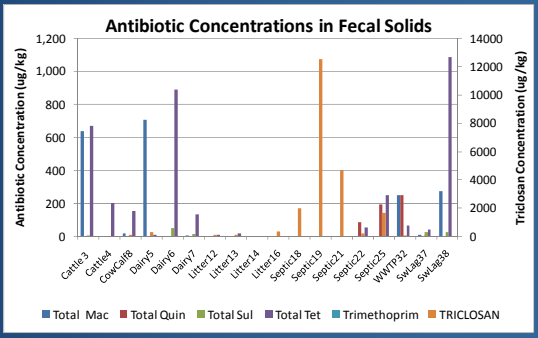
Orchards and some vegetables may be sprayed with antibiotics

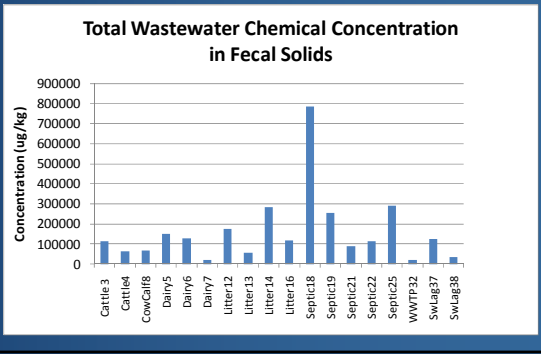
There are numerous chemicals in pesticide and herbicide sprays besides the active ingredients

Contaminant Types and Concentrations Vary by Source

Results from a USGS study

Wastewater chemicals include detergents, phenols, and triclosan to which bacteria develop resistance





Septic System Chemicals

ANTIBIOTICS (Range for 9 samples, µg/L)

Chlortetracylcine	<0.01 for all
Iso-Chlortetracyline	<0.01 – 0.292
Iso-Epi-Chlortetracyline	<0.01 - 0.033
Anhydro-Chlortetracyline	<0.01 for all
Tetracycline	<0.01 - 20.0
Epi-Anhydro-Tetracycline	<0.01 - 1.11
Epi-Tetracyline	<0.01 - 3.81
Ofloxacin	<0.005 – 2.31
Ciprofloxacin	0.036 - 0.270
Erythromycin	<0.01 – 0.140

WASTEWATER CHEMICALS (Example from 1 system)

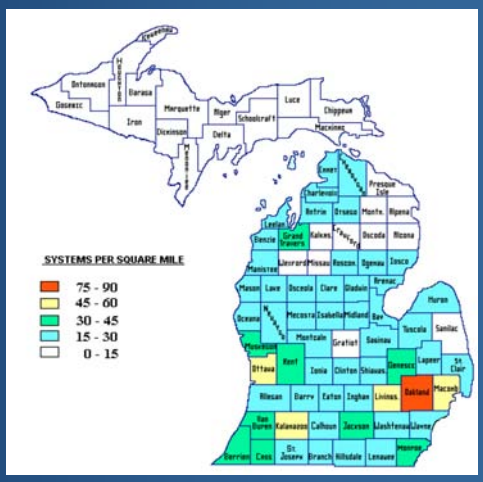
	Liquid (µg/L)	Solid (µg/kg)
AHTN (tonalide)	ND	18000
Triclosan	2.0	19000
p-nonylphenol	18	55000
Carbamazepine	0.05	
Caffeine	38	
Acetaminophen	45	
Cotinine	1.7	

Kolpin, D.W., 2006, Keynote address for "Risk Assessment of Pharmaceuticals & Veterinary Medicines in the Environment, "Sept. 26-27, Amsterdam, The Netherlands

Septic Systems in Michigan

MDEQ, 2001

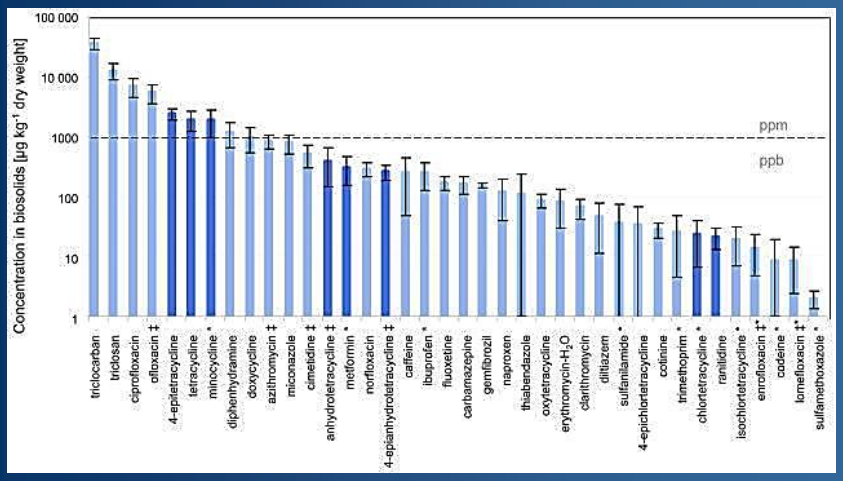
- **1.2 million septic systems in Michigan**
- **Up to 10% are failing**



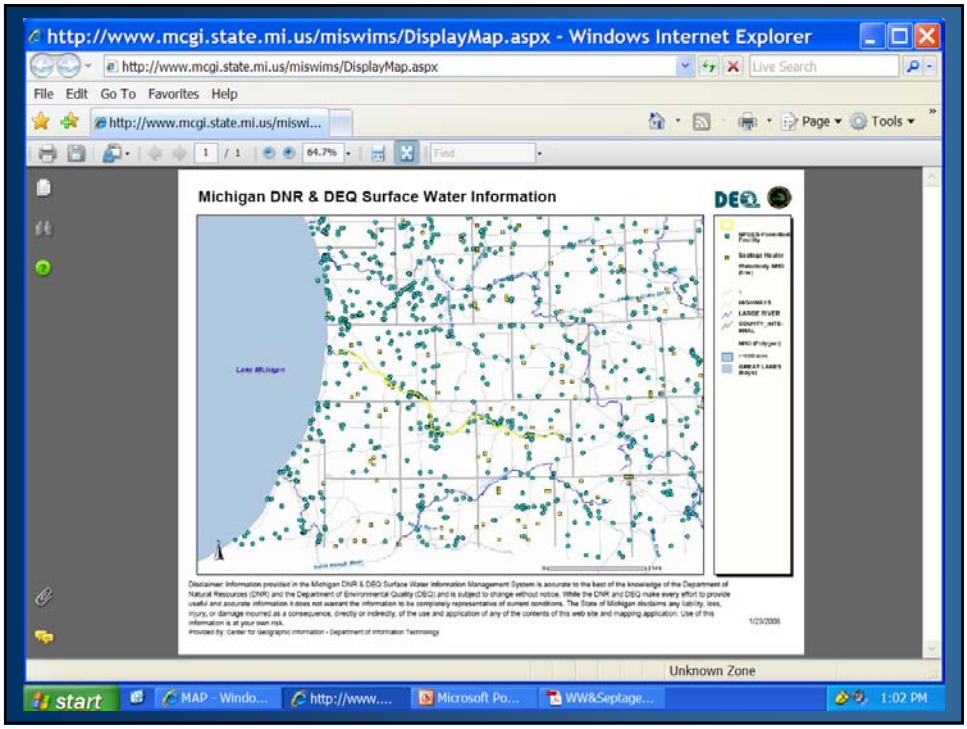
USEPA Targeted National Sewage Sludge Surveys

- 2006/2007: 74 POTWs in 35 states, 72 chemicals
  - ciprofloxacin, diphenhydramine, triclocarban, and flame retardants in all samples
  - hormones in few samples
- 5 composites of 2001 samples archived from 94 POTWs
  - Triclocarban (36 mg/kg) and triclosan (12.6 mg/kg) most abundant
  - Multiple antibiotics, ibuprofen, carbamazepine, other pharmaceuticals detected

USEPA Results for Archived Biosolids



McClellan and Halden, 2010, Water Research 44:658-668

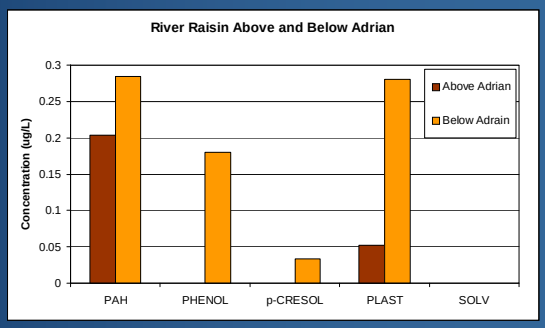


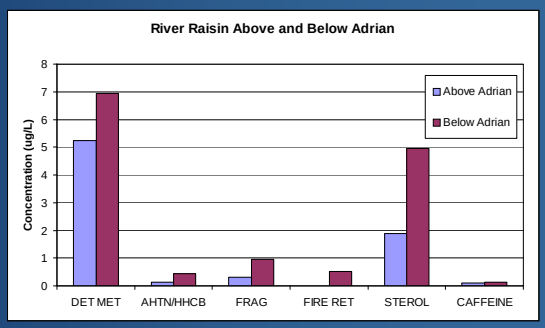
River Raisin Above and Below Adrian

All urban contaminants are in greater concentration downstream of Adrian

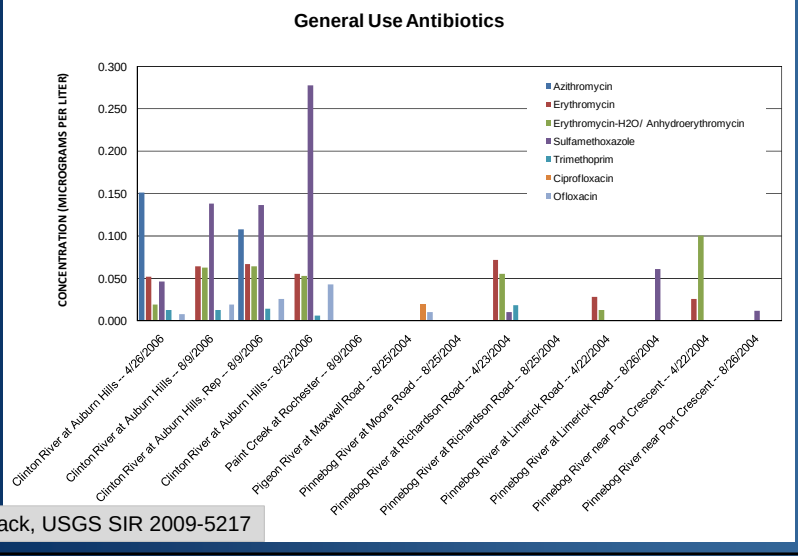
- **Septic**
- **Urban runoff**
- **WWTP**

Haack, USGS SIR 2009-5217





Antibiotics in Water in Oakland and Huron Counties



Haack, USGS SIR 2009-5217

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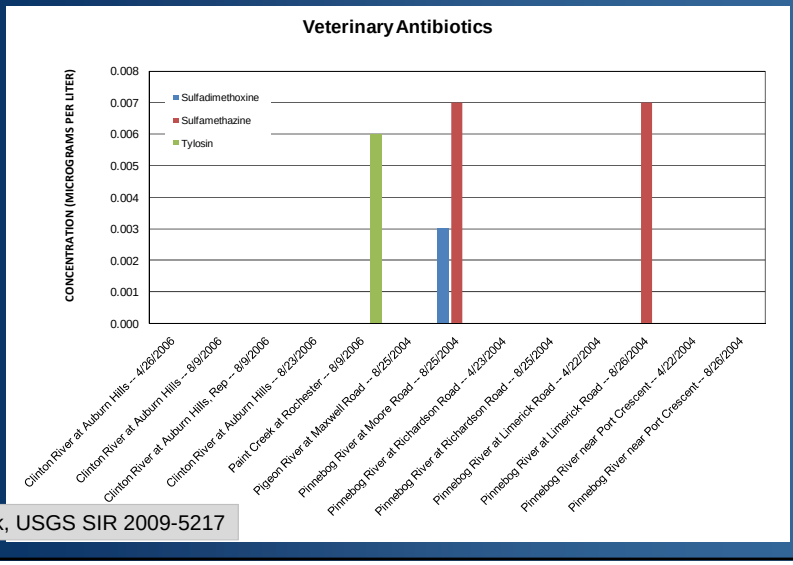


Haack, USGS SIR 2009-5217

ANTIBIOTIC-RESISTANT BACTERIA IN MICHIGAN WATER

2003 Study in Oakland County

- *E. coli* resistant to one or more ß-lactam antibiotics were detected
  - Two of 15 contained integrons
  - Two of 15 were also resistant to gentamicin
- Enterococci resistant to high levels of vancomycin were identified as *E. faecium*.
  - Two isolates carried the *VanA* gene
- A follow-up study failed to detect vancomycin-resistant enterococci, but did detect the *esp* gene (human pathogenic enterococci) in water samples

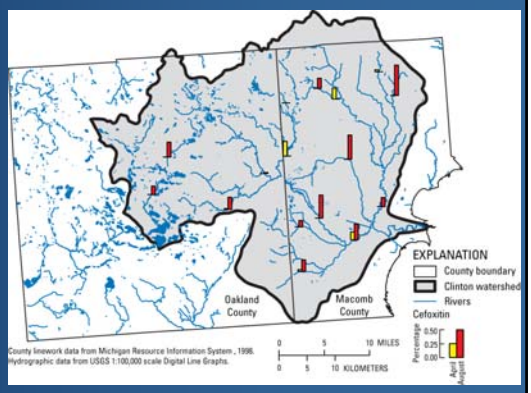
Fogarty et al. USGS SIR 2005-5058

Follow-up Study of Cephalosporin-Resistant *E. coli*

- **ß-lactam antibiotics**
- **Four generations of cephalosporins**
  - **Each generation greater effectiveness against gram-negative bacteria**
    - Cefoxitin- 2nd generation human and vet medicine
    - Ceftriaxone-3rd generation human only use
    - Similar antibiotics have animal usage
- ***E. coli* are not inherently resistant to cephalosporin antibiotics**

Spatial Distribution of Cefoxitin-Resistant *E. coli* in the Clinton River Watershed

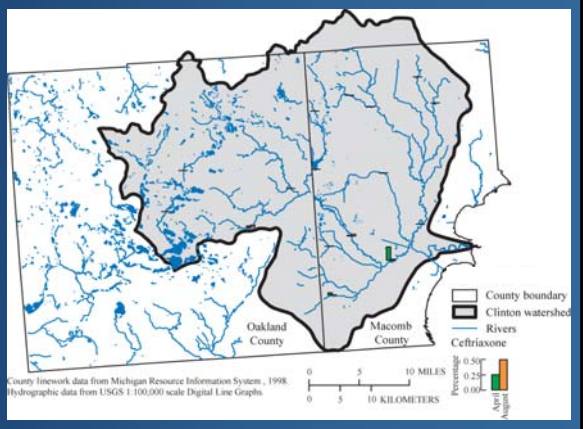
- **50 isolates from each site on each date tested for resistance using clinical resistance criteria**
- **Two sites had no detections**
- **Sites with largest percentage resistant were very urban, or very agricultural**



Fogarty et al., USGS SIR 2007-5242

Spatial Distribution of Ceftriazone-Resistant *E. coli* in the Clinton River Watershed

- Ceftriazone resistance far less prevalent
- Detected in urban/industrial areas



Fogarty et al., USGS SIR 2007-5242

Dairy Manure Application, Lenawee County

- Study of effect of application rate and method on tile drain water quality
- Small ancillary study of *E. coli* isolated from manure, and from a drain 48 hours and 26 days following land application

Percent of 96 Isolates Resistant			
Antibiotic	Lagoon Waste	48 Hour Tile	26 Day Tile
SUL	81	100	100
ERY	84	100	100
TET	39	96	41
AMP	78	77	97
SE	84	100	100
SET	36	96	41
SETA	34	74	39
SETA-CEP	2	0	10
SETA-CFX	0	5	0

Enterococci from Gulls, Michigan Beaches

Enterococci exhibiting high level resistance to streptomycin, gentamicin and tetracycline were isolated from gull feces at Grand Traverse Bay beaches

No gull enterococci displayed intermediate or high level resistance to vancomycin

Haack at al, 2003, ES&T 37: 3275-3282

Summary

Environmental studies demonstrate that bacterial pathogens can be detected in water with which Michigan residents have contact

- Typical tests for fecal indicator bacteria are not necessarily predictive of pathogen occurrence
  - National consensus on this issue
- Water contact should be considered as a possible exposure route for the typical "foodborne" pathogens (*E. coli*, *Salmonella*, *Campylobacter*)

Summary

Human-use chemicals, including antibiotics, pharmaceuticals, and a variety of chemicals to which bacteria develop resistance can be detected in the Michigan environment

Sources are abundant

- **Not unique to Michigan**
- **Water concentrations usually low**
  - Wide array of chemical mixtures
  - May occur with “traditional” pollutants, such as pesticides or industrial chemicals

Summary

Antibiotic-resistant bacteria occur in Michigan waters

- **MRSA**
- **Vancomycin-resistant enterococci**
- **Third-generation ß-lactam resistant *E. coli***

Sources are likely human and animal wastes

Needs further study to determine significance