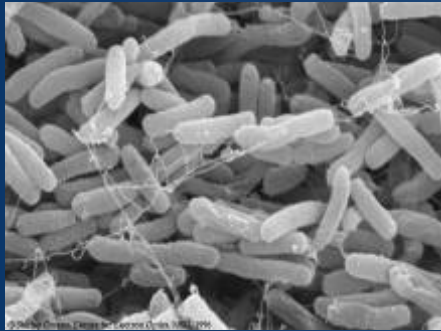# *E. coli* and Bacterial Pathogens Studies – Summer 2010
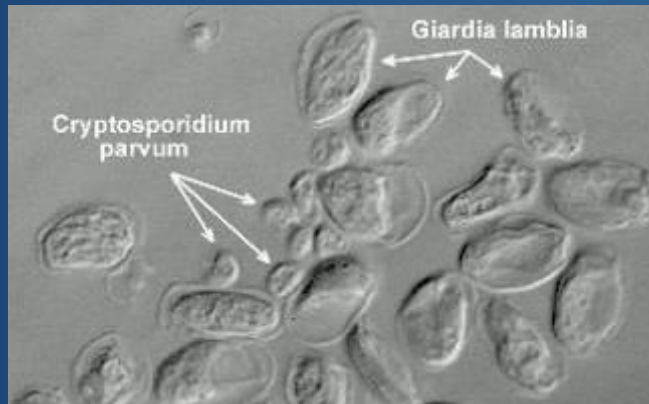
Sheridan K. Haack, Lisa R. Fogarty, Chelsea Spencer, Angela K. Brennan, Natasha Isaacs-Cosgrove, Heather E. Johnson,

Great Lakes RESTORATION

≋USGS
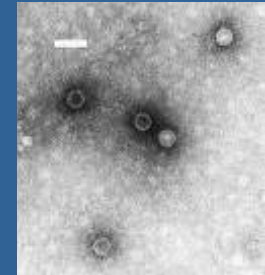*science for a changing world*

E. coli bacteria

Protozoa

Poliovirus

FECAL INDICATOR BACTERIA AND PATHOGENS

Pathogens

Cause human disease

- **Bacteria** (some can be transferred from animals)
    - *E. coli* O157:H7, *Campylobacter jejuni*, *Salmonella*, *Shigella*, *Staphylococcus*
- **Protozoa** (some can be transferred from animals)
    - *Cryptosporidium*, *Giardia*
- **Viruses** (most viruses of human-health concern come from humans)
    - Norovirus, rotavirus, hepatitis A virus, adenovirus

Every pathogen requires a different test

Every pathogen behaves differently in the environment

So…How Do We Evaluate Microbial Pollution?

Water must be free of "fecal pollution"

- Not necessarily free of pathogens

How do we define "free of fecal pollution"?

- Quantify the numbers of "fecal indicators" and relate these to disease in epidemiological studies

What are the "fecal indicator bacteria"?

- *E. coli* and enterococci for beaches
- These organisms are indicators—they are not the problem

	E. coli per 100 mL	Enterococci per 100 mL
Any single sample	235	61
Over 5 days (geometric mean)	126	33

What Are Fecal Indicators Intended to Indicate?

Feces are in, or have recently been introduced to, the water

Pathogens in those feces may cause disease in swimmers

Problems with fecal indicator bacteria

1. They may grow in the environment and therefore don't indicate fecal pollution
2. They die faster in the environment than some other types of pathogens (e.g., viruses)
3. They cannot represent non-fecal pollution

Our Goals

Test *E. coli* more frequently (4 days per week)

- Does the high quality of Ottawa County beaches stand up to more frequent testing?

Obtain environmental data to relate to *E. coli* concentrations

- Can we predict *E. coli* concentrations?

Test for bacterial pathogens

- Are bacterial pathogens present?
- Are they related to *E. coli* concentrations?
- Are they related to environmental conditions?

E. COLI AT OTTAWA COUNTY BEACHES

E. coli and Environmental Sampling

Monday-Thursday samples

- **Health Department**
- **USGS**
- **Alliance For The Great Lakes**

***E. coli* by IDEXX**

Daily environmental records

- **Numbers of swimmers, birds, dogs; wave height, current direction; rating of algae, debris, etc.**
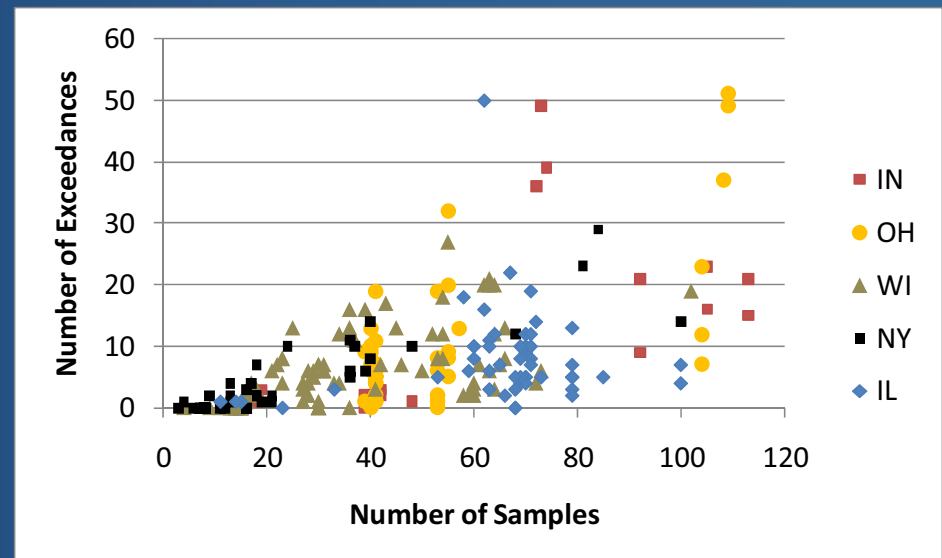
Online database

Ottawa County Beaches Are Astoundingly Clean of *E. coli*

2008

About 40-50 *E. coli* analyses per beach summer of 2010

Grand Haven and North Beach had no exceedances of *E. coli* standards

Can't predict exceedances if there aren't any!

Few beaches in the Great Lakes can be sampled more than 40 times and not have exceedances of *E. coli*

PATHOGENS AT OTTAWA COUNTY BEACHES

Bacterial Pathogens of Interest

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

Bacterial Pathogens of Interest

Staphylococcus

- Arise from the skin of humans
- Known to be present in Great Lakes swimming waters since the 1980's
- Recent study indicated humans shed 1,000,000 staphylococci in 15 minutes of water exposure
- Not fecal source so *E. coli* not an indicator

Methicillin-resistant *Staphylococcus aureus* (MRSA) recently reported from marine beaches

General Study Approach

27 samples each from Grand Haven State Park and North Beach

- Collected by USGS student under a range of environmental conditions
- Composites of the three beach *E. coli* sampling locations
- Shipped overnight to USGS lab
- Processed day of receipt
- Tested for pathogen genes

Staphylococcus Studies

Grand Haven State Park Beach, North Beach, and 10 other Great Lakes beaches were sampled for *Staphylococcus* (all types) about 25 times each under various conditions

At Grand Haven State Park Beach, samples were collected over the course of a day

- **6 beach sampling sites**
- **8 AM, 10 AM, noon, 2 PM and 4 PM**
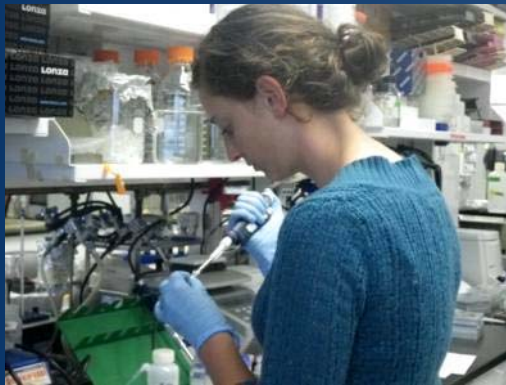- **environmental variables recorded**

Methods

Remove growth from plate and preserve by freezing

Extract DNA from **all** organisms that grew and test for bacterial pathogen genes
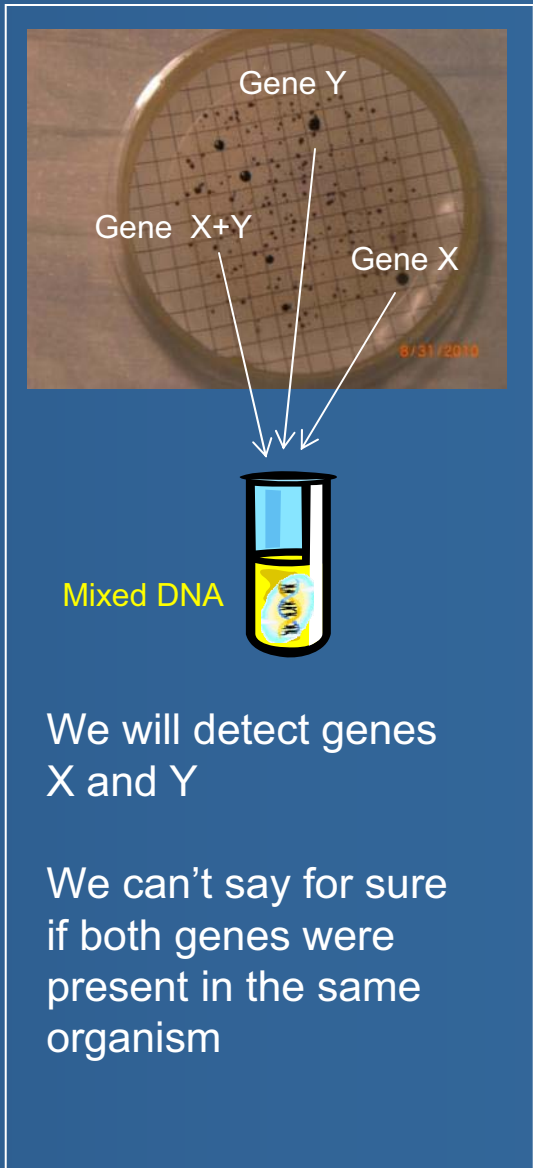
Important Aspects of Our Assays

Methods are similar to those used to test for pathogens in hospitals

Since the tests are on organisms that grew, we know they are viable

We know there was at least one of the target organism in the 100 mL sample

However, we do not have isolated organisms by this procedure

Gene Y

Gene X+Y

Gene X

Mixed DNA

We will detect genes X and Y

We can't say for sure if both genes were present in the same organism

Summary of Assays

Shiga-toxin producing *E. coli* genes tested:

- *eae*A, *stx*2, *stx*1, *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*

***Staphylococcus* genes tested**

- *fem*A (*Staphylococcus aureus*)
- *mec*A (methicillin resistance)

GENERAL PATHOGENS - PRELIMINARY RESULTS

Beach Characteristics

Beach	Number of Samples	Land Use	E. coli Density (Exceed)*	Enterococci Density (Exceed)*	Average Number of Swimmers	Average Number of Birds	Average Turbidity (NTU)	Average Wave Height (ft)
Lake Huron 2	21	Forest/Wetland	5 (0)	2 (0)	3	18	7	0.21
Grand Haven SP	27	Urban	20 (0)	15 (3)	133	131	10	1.37
North Beach	27	Mixed	18 (0)	10 (2)	1	62	8	1.26
Lake Huron 1	21	AG	146 (4)	93 (15)	<1	1	11	0.34

*Bacterial geometric mean density in CFU/100 mL; single sample exceedances; results for indicated number of samples

At Grand Haven SP and North beach, *E. coli* and enterococci concentrations were low over the course of the summer

If enterococci were used, there would have been some exceedances of water quality standards

Data are preliminary and are subject to change

Pathogen Detections

Beach	Land Use	E. coli Density (Exceed)	Enterococci Density (Exceed)	Average Number of Swimmers	All Samples*			Subset of Samples		
					Shigella	Campylo-bacter jejuni/ coli	E. coli O157	Shiga toxin 2	Shiga toxin 1	E. coli O157 + stx 2 + stx 1
Lake Huron 2	Forest/Wetland	5 (0)	2 (0)	3	0	1	2	2/7	2/7	0/7
Grand Haven SP	Urban	20 (0)	15 (3)	133	1	1	3	0/14	0/14	0/14
North Beach	Mixed	18 (0)	10 (2)	1	2	0	2	2/14	2/14	1/14
Lake Huron 1	AG	146 (4)	93 (15)	<1	0	3	5	1/7	2/7	0/7

*21-27 samples

Some pathogen genes were detected at every beach sampled

Until all genes, all samples, all beaches are analyzed it is difficult to draw any conclusions

Data are preliminary and are subject to change

Summary of General Pathogen Results

No clear relation between indicator concentrations and pathogen detections so far

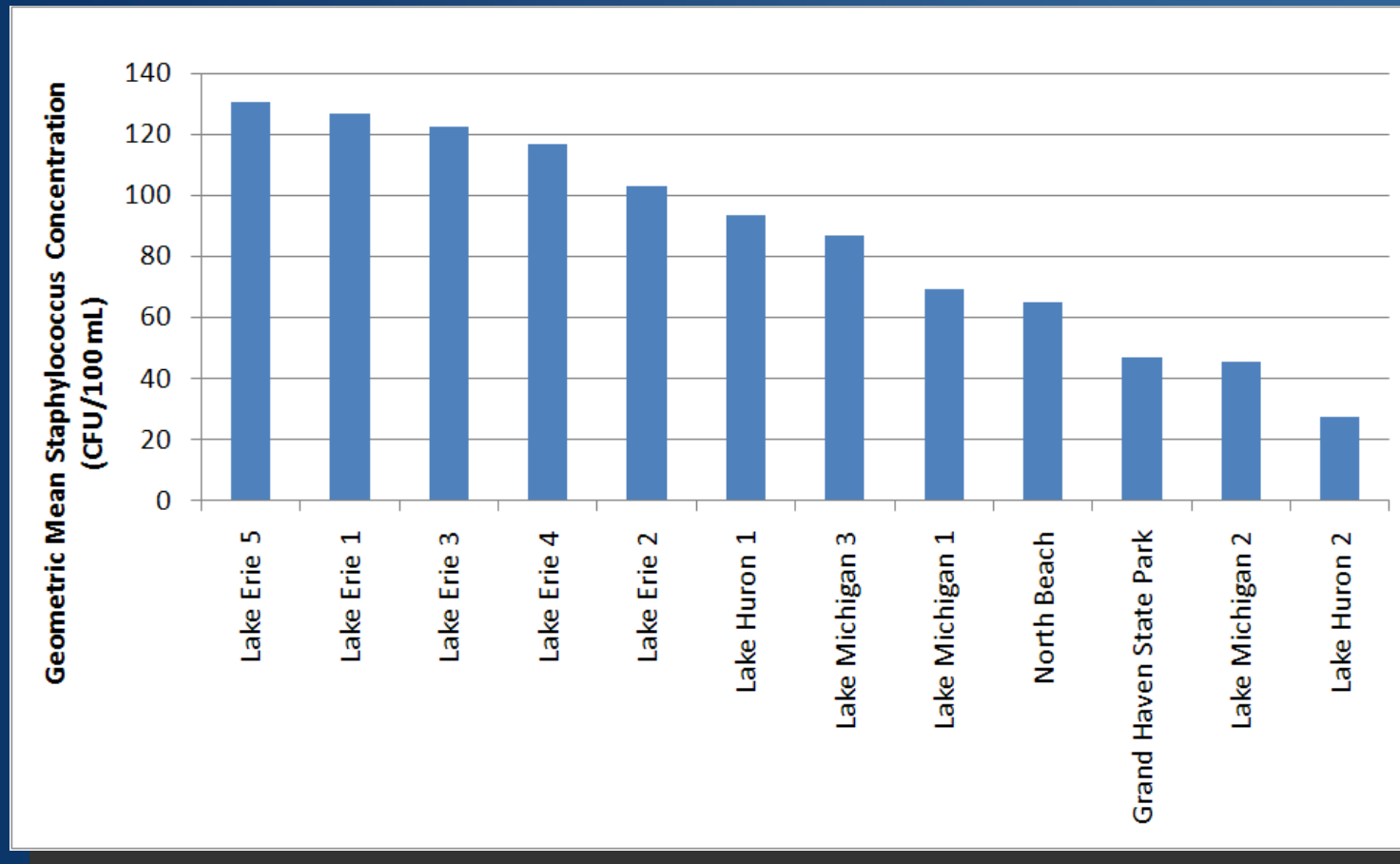No clear relation between *E. coli* and enterococci exceedances so far

At Grand Haven SP, no environmental factor could be related to pathogen gene detections

At North Beach, bird concentrations were implicated for some detections

PRELIMINARY
STAPHYLOCOCCUS RESULTS

Staphylococcus Were Detected at All Beaches

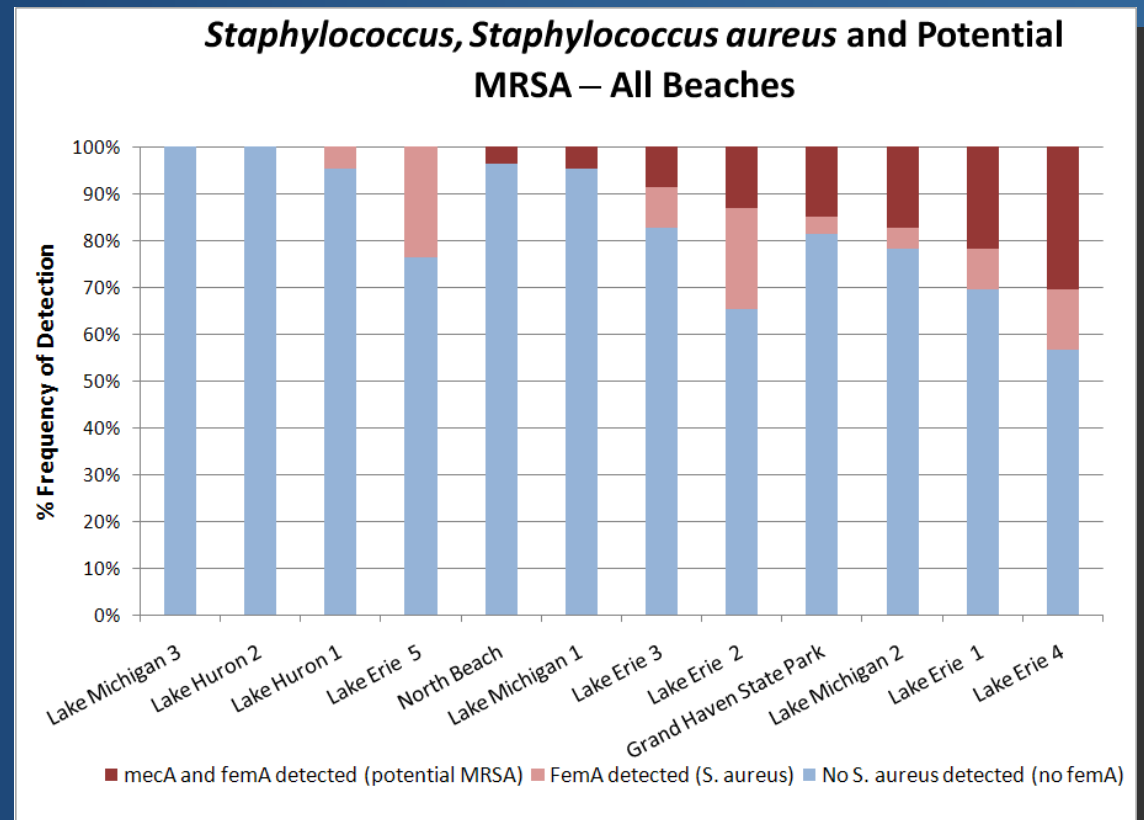Ottawa County beaches were in the lower range of concentrations of all beaches tested

Data are preliminary and are subject to change

Staphylococcus aureus and Potential MRSA Were Also Detected at Most Beaches

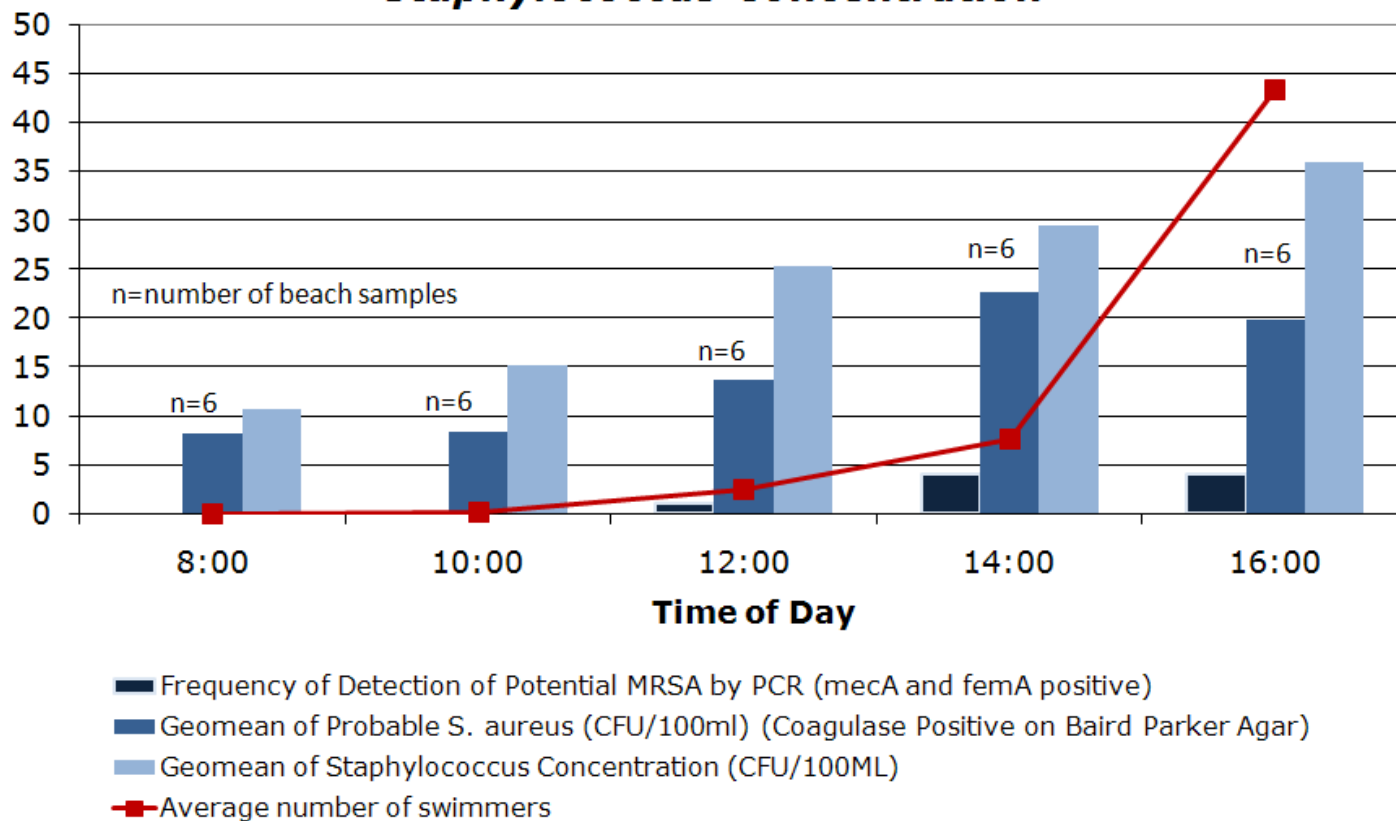Remember —

No MRSA organism was isolated

We do not at this time have absolute proof that MRSA organisms carrying both genes were present

Staphylococcus, Staphylococcus aureus and Potential MRSA — All Beaches

% Frequency of Detection

Lake Michigan 3, Lake Huron 2, Lake Huron 1, Lake Erie 5, North Beach, Lake Michigan 1, Lake Erie 3, Lake Erie 2, Grand Haven State Park, Lake Michigan 2, Lake Erie 1, Lake Erie 4

■ mecA and femA detected (potential MRSA) ■ FemA detected (S. aureus) ■ No S. aureus detected (no femA)

Grand Haven State Park Special Study

Staphylococcus aureus and Potential MRSA Increased with Number of Swimmers and Staphylococcus Concentration

Summary of *Staphylococcus* Studies

Staphylococcus occurred at all tested beaches across the Great Lakes

S. aureus is suggested by gene-based studies at the majority of Great Lakes beaches

MRSA may be present at Great Lakes beaches – needs much more study

At Grand Haven SP beach, swimmers were implicated as the source of *S. aureus*

- *E. coli* and enterococci do not indicate *Staphylococcus*

Next Steps

Complete all pathogen analyses

Evaluate relations among pathogen detections and environmental conditions

- **At each beach**
- **Among all beaches**

Evaluate relations among pathogen detections and indicator bacteria concentrations

- **Evaluate models**

Follow up selected samples with

- **qPCR for pathogen genes**
- **Microbial source-tracking**

Summary

Ottawa County beaches have very low concentrations of *E. coli* in comparison to other Great Lakes beaches

- May mean low levels of fecal pollution

But, bacterial pathogens are indicated at Ottawa County beaches by gene analyses

- There is no obvious relation to *E. coli* or enterococci concentrations
- Perhaps not a fecal source?
    - This is definitely true for *Staphylococcus*

QUESTIONS?