

Use of New Tools for Managing Risks Microbial Source Tracking

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Microbial Source Tracking

- Tools are now available to determine the molecular fingerprint of the fecal pollution.
- Health risks
- Remediation
- Prioritization
- Responsibility



Microbial Source Tracking

- Indicator bacteria *E.coli* don't provide source of pollution
- MST methods have developed using genetic approaches to identify source.
- Can be library dependent or library independent/host specific

Why host specific ?

- Host specific method is library independent
- For Library-dependent, DNA libraries are built using isolates from animals & human sources in the area
- Library dependent methods use these libraries to match to the unknown environmental sample
- Host specific method requires no library, the marker is specific to the host, and has fewer false(-) and false(+) the library methods

What are Fecal Indicators?

- A microorganism that...
 1. is present when feces and presumably pathogens are present (health risk)
 2. occurs in high concentrations in polluted waters and has a relationship to the degree of pollution
 3. is easy to cultivate and identify

E. coli

- Part of the fecal indicator bacteria which are generally harmless themselves
 - Found in high numbers in the gut of humans and other warm blooded animals, including birds
 - Excreted daily in the feces of people and mammals
 - Used to indicate the potential presence of pathogens, microorganisms that come from the gut and cause diseases such as diarrhea



Where are fecal indicators found?

- Sewage (even treated)
- Septic tank effluent (the liquid from the septic tank)
- Septage (solids from the septic tank)
- Manure and animal waste lagoons
- Bird and other animal droppings
- Rainfall can wash the fecal wastes and associated indicator bacteria into nearby water bodies
- Almost all surface waters have some background of fecal indicators due to wildlife, with the levels increasing the closer you get to sewage/feces

Water quality Standards/Criteria For Recreational Water

Indicator Geometric Means	Michigan	EPA
E.Coli CFU	<130/100ml	<126cfu/100ml
Enterococci CFU	None	<33cfu/100ml

Our Approach: host-specific markers

- Use multiple source tracking markers
- Host specific genetic marker (esp)
 - Differentiate Human/non human pollution
- Traditional MF method and PCR used to detect the gene. A culture dependent library independent method
- Bacteriodes: use PCR

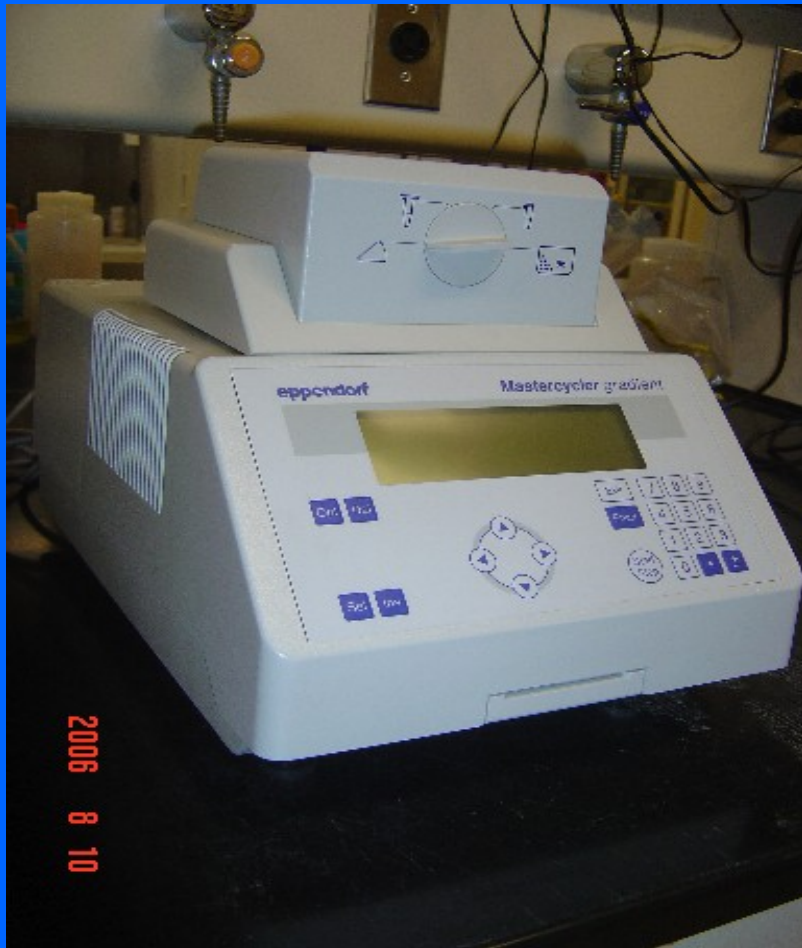
What is PCR?

- Enzymatic reaction that makes many copies of DNA from single molecule
- 2^n copies of DNA from single molecule where $n = \text{No. of cycles}$
- So, 35 cycles of PCR would yield 2^{35} copies of DNA

Polymerase Chain Reaction (PCR)

- Developed in 1985 by Kary Mullis
- Dr. Mullis received the Nobel Prize in Chemistry in 1993
- PCR is considered as one of the most important discoveries in molecular biology

PCR Machine



Enterococci

- EPA recommended
- Esp (Enterococcus surface protein) as human marker
- Only found in human feces
- Cause of urinary tract and gastrointestinal infection

Enterococcus membrane filtration (MF)



**Colonies that
have a blue
halo are
counted as
enterococci.**

BACTERIODES

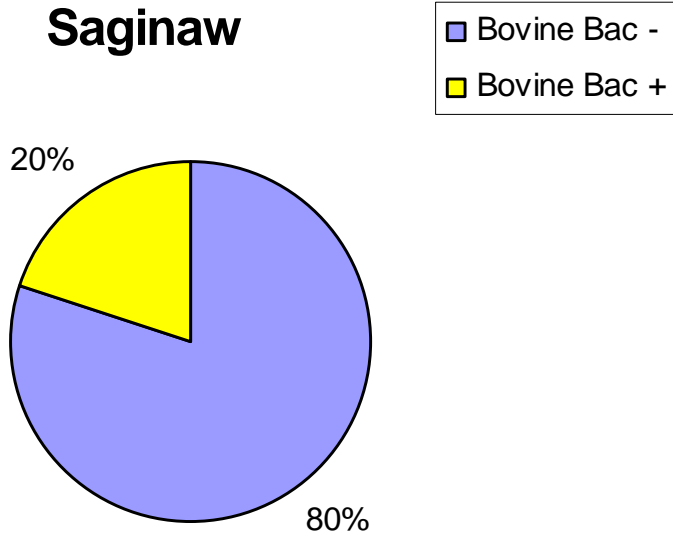
- *Bacteroides* are specialists in the intestinal environment of animals.
- Are the most numerous types of bacteria approximately 30 % of what is excreted.
- These bacteria are anaerobes and can not grow in the presence of oxygen.
- Specific genetic targets have been identified in cattle, humans and swine.

Host Specific Markers are Key to Source Tracking Future

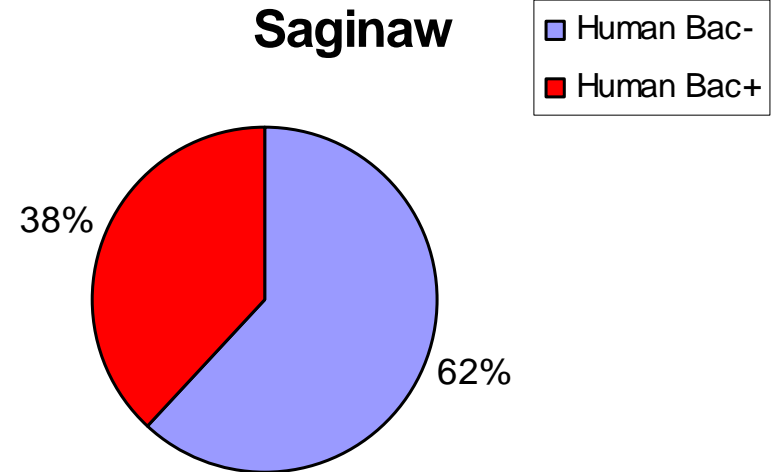
- Bacteroides (genetic approaches PCR)
- 4/4 sewage; 4/4 human; 4/5 cow (lowest concentration missed) 4/4 dogs however no marker for Birds: Missed 2 samples with dog and 2 with cow that were mixed.
- E.coli Toxin genes able to detect sewage (4/4).
- Enteroviruses and Adenoviruses found in 3 of 4 sewage samples.
- Enterococci ESP marker found in 100% of 109 human sewage water samples and zero of 80 animal samples.

Prevalence of Human and Bovine Markers

Saginaw

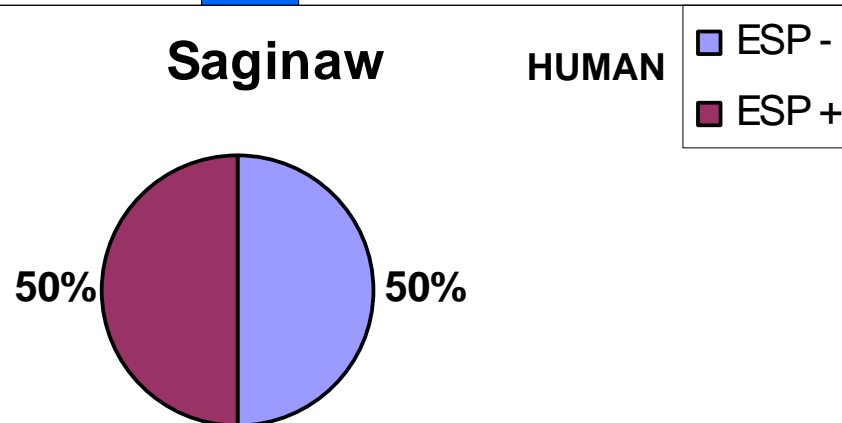


Saginaw



Saginaw

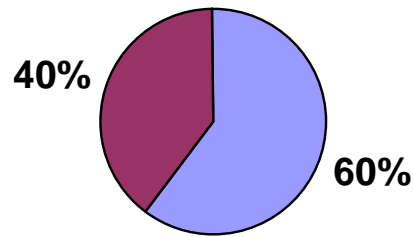
HUMAN



Prevalence Of esp + Human marker

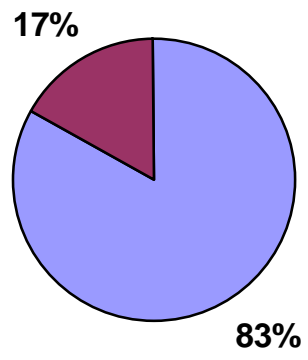
Silver Lake

ESP -
ESP +



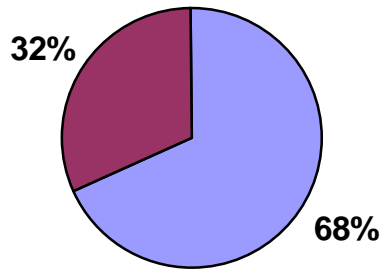
Silver Beach At Lake Michigan

ESP -
ESP +



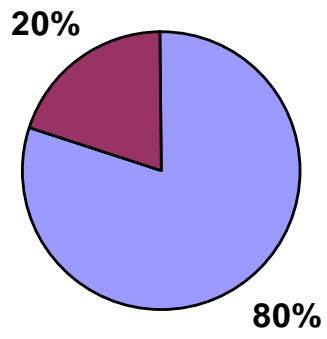
Washinton Park At Lake Michigan

ESP -
ESP +

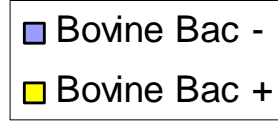
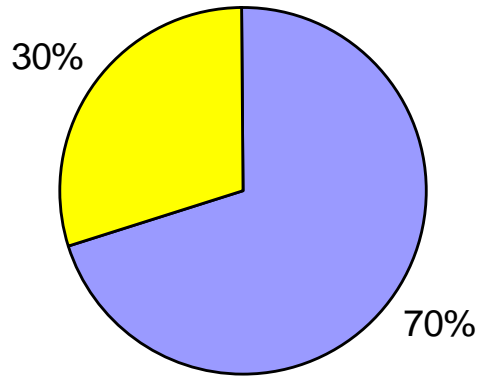


Grand River

ESP -
ESP +

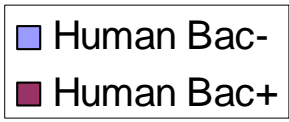
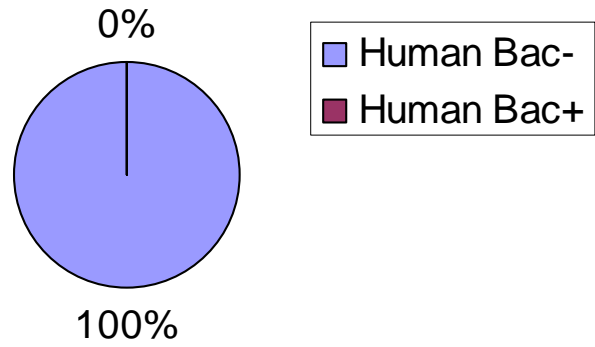


Coldwater Creek

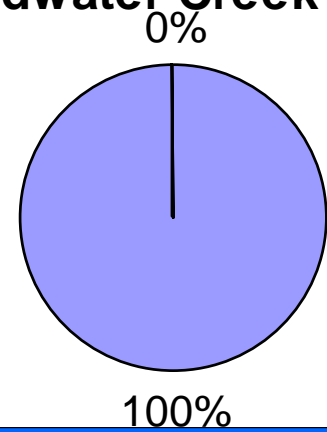


Prevalence Of Human And Bovine Markers

Coldwater Creek



Coldwater Creek



Conclusions

- New tools allow for the detection of multiple sources of pollution using a tool box approach.
- This will help address the types of pathogens of concern.
- This will also help to determine type of future controls on land that will protect water quality.
- More samples need to be collected and assayed in the future.

THANK YOU

