Microbial source tracking: using old and new technologies to find out what is in our water

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Introduction

- Background
- Goals
- Sites
- Methods
- Results/Summary
- Future work and next steps

Snow Geese flyway

North Inlet Beach
Fecal indicators of contamination

- FIB –
  - Non-pathogenic bacteria found in fecal waste
  - Proxies for potential enteric pathogens
  - Threshold determined by quantifiable relationship between:
    - Density of indicator bacteria
    - Health risk to those using the water

<table>
<thead>
<tr>
<th>Fecal Indicator Bacteria</th>
<th>Recommended by US EPA in:</th>
<th>Threshold for contamination (colonies/100 mL)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal coliforms</td>
<td>1976</td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>Total coliforms</td>
<td>1976</td>
<td>2400</td>
<td></td>
</tr>
<tr>
<td>Total Enterococcus</td>
<td>1986</td>
<td>104</td>
<td>Used by State of Delaware</td>
</tr>
<tr>
<td>E. Coli</td>
<td>1986</td>
<td>235 (freshwater only)</td>
<td>Not recommended for marine waters</td>
</tr>
</tbody>
</table>
Fecal indicators, continued

- **Total *Enterococcus*** –
  - Used by Delaware DNREC to determine water quality
  - Guarded beaches
    - Sampled weekly during summer
    - Closed when exceed threshold
  - Non-recreational waters
    - Sampled monthly
    - Historical purposes
Map of monitoring sites as percentage of samples that exceeded the single sample primary recreational water contact standard of 104 Enterococcus cfu/100 mL in the Delaware Inland Bays from 2004 to 2008. Legend: Green = 0 – 10%, Yellow = 10-25%, Orange = 25-75%, red = 75-100%.

From:
Delaware Center for the Inland Bays Environmental Indicators Series 2009-2010, Development of the Recreational Water Quality Indicator

Chris Bason
Human Pathogens in the environment

- Detection
  - Culture-based approaches
  - PCR*
  - Microarrays
- Activity
  - Not yet assayed
- Quantification
  - qPCR-based approaches
- Relationship to epidemiology

* See next slide for explanation
What is PCR?

- Isolates and amplifies a section of DNA

http://schoolworkhelper.net/2011/06/pcr-uses-steps-purpose/
Pathogen-like *Epsilonproteobacteria*

- **Helicobacter spp.** –
  - *Helicobacter pylori*
    - Asymptomatically colonizes guts of 20-80% human population in developed countries
    - Causative agent of:
      - Gastritis
      - Peptic ulcers
      - Gastric cancer
    - Presence/absence of virulence factors
  - Transmission route unknown – believed to be fecal-oral
  - Found in VBNC state and associated with zooplankton and particles

Electron micrograph of *H. pylori*
www.health.qld.gov.au
Goals of Project

- Detection of FIB and *Epsilonproteobacteria* in environment, June 2007-August 2008
  - Human-specific *Bacteroidetes* spp.
  - *Helicobacter pylori*, pathogenic *H. pylori*

- Correlation to traditional indicators of fecal pollution
- Correlation to environmental conditions

- Master’s student, Katrina Twing
Sites studied
Methods

- Environmental parameters
  - Temp., salinity, DO, chlorophyll a, NO₃, NH₄, PO₄, DO
- Measure FIB via traditional methods (TE counts)
- Measure FIB using molecular methods (PCR with human-specific *Bacteroides* primers)
- Detect *Epsilonproteobacteria* via PCR (whole water)
  - *Helicobacter* spp. primers
  - *Helicobacter pylori* primers
    - 16S
    - cagA (pathogen-specific)
Results

- Approx. 10% samples exceed recommended FIB limit
- Less than 4% are positive for human *Bacteroides*
- About 5% are positive for *Helicobacter pylori*

N=145
Sites studied - results

- TE >104
- Human Bacteroides spp.
Results by site type

- Coastal Beach: TE >104 (N=31), Human Bacteroides spp. (N=5)
- River: TE >104 (N=28), Human Bacteroides spp. (N=75)
- Inland Bays: TE >104 (N=75), Human Bacteroides spp. (N=11)
- Delaware Bay: TE >104 (N=11), Human Bacteroides spp. (N=11)
Sites- *Helicobacter pylori*
Results by site type

- Coastal Beach: Helicobacter spp. (N=31)
- River: H. pylori (N=28)
- Inland Bays: H. pylori cag A (N=75)
- Delaware Bay: Helicobacter spp. (N=11)

Legend:
- Blue: Helicobacter spp.
- Turquoise: H. pylori
- Light turquoise: H. pylori cag A
Relationship to environmental parameters

- TE counts >104
  - Low salinity
- Human *Bacteroides* spp.
  - Low nitrate/nitrite
- *H. pylori*
  - High salinity
  - Low chlorophyll a

![Graph showing presence/absence of Helicobacter, H_pylori, and Cag_A across salinity levels.](image-url)
Summary – past work

- No correlation between TE counts and the PCR detection of human *Bacteroides* or pathogenic *Epsilonproteobacteria*.
- *H. pylori* and pathogen-specific *H. pylori* less prevalent, ranging from 3-9%; mostly in coastal and DE bay sites.
- % positives were much greater when separated by year.
What’s next?

PCR based approaches are sensitive and specific, but...

• We really want to know what the source of the contamination is – is it human or animal?
  • Microbial Source Tracking
  • Use of high throughput sequencing approaches for detection and design of new quantitative PCR primers

• We really want to know how much is there and if it is viable.
  • Quantitation of contaminant by PCR from RNA
Microbial Source tracking

Sources of fecal pollution are typically mixed, but may arise mainly from a single source. Figure adapted from Figure 1 in (Roslev and Bukh 2011).
Microbial Source tracking

• **Purpose**
  • Determine the source of fecal contamination
  • Molecular methods (do not rely on culturing)
  • Indicative of potential pathogens
  • Host specificity

• *Bacteroides* spp.
  • Distinct genetic variation among bacteria found in different hosts
  • Strong correlation with enteric pathogens
    • Specifically *Campylobacter* spp.
  • Can not survive for long periods of time outside of host
High throughput sequencing

- **Purpose**
  - Amplify a region of the genomes of all bacteria (ribosomal RNA or ribosomal RNA gene)
  - Produce thousands to millions of sequences from a sample*
  - Analyze the data to characterize the types of bacteria in the sample
  - Can discriminate between sources of contamination, if enough known about the bacteria [sequences] found in different sources
  - Work with Jorge Santo Domingo at EPA who has collections of fecal material from animals

- **Cost effective**
  - Now generally less than $100 a sample

* See next slide for images and information on HTS machines
Roche 454 machine

400+ bp read length

Can multiplex easily

Output ~1 million reads/$10,000
Illumina HiSeq machine
100+ bp read lengths
Can multiplex easily
Output ~100 million reads/$1,000
High throughput sequencing

- What to do with the data?
  - Design PCR primers to detect and quantitate specific bacteria from different sources
  - Test on known sources, original samples and new environmental samples
Are these bacteria viable?

- Viable bacteria contain both RNA and DNA in cells
- Dead bacteria generally contain only DNA
- Sometimes have ‘naked’ DNA in the sample

- Two molecular ways to test for viability:
  - Use RNA instead of DNA in tests
  - Treat samples to get rid of ‘naked’ DNA prior to cell lysis and extraction
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  - Alison Boyer
  - Edythe Humphries
  - Sergio Huerta
What I would like from you

• Advise on
  • Where to collect samples
  • How often to collect samples
  • Types of potential sources
  • Archived fecal material from other animals?