Lake Granbury Bacterial Source Tracking

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Presentation Outline

- Overview of *E. coli* bacterial source tracking (BST)
- *E. coli* BST results
- Overview of *Bacteroidales* BST
- *Bacteroidales* BST results
- Identification of likely sources of pollution
- Discussion

Solving Water Quality Problems

**Clean Water Act**: Restore and maintain beneficial uses of water bodies

**Texas Water Quality Criteria**

*E. coli* - indicator of fecal pollution and health risk

- Long-term geo. mean ≤ 126 CFU/100 ml

274 waterbodies in Texas listed as bacterially impaired on 2008 303(d) list
Fecal Pollution - What to Track?

- **Chemicals**
  - Caffeine, Brightening Agents, Fecal Sterols

- **Viruses**
  - Human, Animal, Bacterial

- **Parasites**
  - Cryptosporidium

- **Bacteria**
  - Bacteroidales
  - *E. coli*
  - Enterococcus

There Are *E. coli* in the Water, But Where Did They Come From?

- Develop and Implement Best Management Practices (BMPs)
- Bacterial Source Tracking (BST) as a tool

Track fecal pollution sources using *E. coli*
- Different animal guts → Different adaptations → Different *E. coli* strains → Genetic Differences → Phenotypic Differences
There Are *E. coli* in the Water, But Where Did They Come From?

- BST - laboratory tests to determine if *E. coli* (or other fecal bacteria) in water samples came from animal or human feces

- Most BST methods are Library Dependent
  - Need database of reference bacteria from known animal and human sources

- “Local” watershed libraries currently considered most useful
  - Cost and time considerations

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Approach

Isolation of *E. coli* From Source and Water Samples

- *E. coli* isolation from samples using same media for compliance water monitoring
  - USEPA Method 1603 – modified mTEC medium
  - Confirmation of β-D-glucuronidase activity of isolates using NA-MUG
  - No broth enrichment or clinical media - avoid selecting different populations of *E. coli*
Isolation of \textit{E. coli} From Feces and Water

- **Fecal Specimens**
- **Modified mTEC Medium**
- **Water Sample Filtered and Filter Placed on Modified mTEC Medium (EPA Method 1603)**
- **E. coli Colonies**

Each \textit{E. coli} colony is an "isolate"

Purification and Confirmation of \textit{E. coli}
Archival (Freezing) of *E. coli* Isolates

- Isolates stored frozen at -80 °C (-112 °F)
- Remain alive for years
- Living library of isolates can be shared with other researchers

**BST Technique 1**
**ERIC-PCR Fingerprinting**

- Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR)
- Method of generating a DNA Fingerprint for each *E. coli* isolate
- Different strains of *E. coli* have different fingerprints
**BST Technique 2**

*HindIII Automated RiboPrinting*

- Another DNA Fingerprinting Test
- Also Confirms Isolates as *E. coli*

### Data Analysis

**Best Match Approach**

- DNA fingerprints – Pearson correlation curve-based analyses
- “Best Match” approach with minimum similarity cutoff based on laboratory QC data
  - Water isolate must match library isolate ≥ minimum similarity or unidentified
  - Identification to *single library isolate* with highest similarity – max similarity approach
Data Analysis
Best Match Approach

Best ERIC-PCR Match (96.9% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library

Best RiboPrint Match (95.8% Similarity) of Water Isolate to Known Source (Pig) Isolate in Library

No Match (Unidentified) Water Isolate, Best ERIC-PCR Match of only 82.4% Sim Library Isolate

No Match (Unidentified) Water Isolate, Best RiboPrint Match of only 65.9% Sim to Library Isolate
Minimum similarity for match
≥ 80% identical

68% ID rate for 1,592 water isolates from 1,375 samples
**E. coli BST Accuracy**

**Combined Texas Restricted Cross-Validated Library and Lake Granbury Local Library**

**Rates of Correct Classification (RCCs)**

<table>
<thead>
<tr>
<th>Source Class</th>
<th>Lake Granbury Local Library</th>
<th>Combined Texas Restricted Cross-Validated Library and Lake Granbury Local Library</th>
<th>% Random RCC</th>
<th>% RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td># fecal samples</td>
<td># isolates</td>
<td># fecal samples</td>
<td># isolates</td>
</tr>
<tr>
<td>Sewage/Septage</td>
<td>17</td>
<td>21</td>
<td>96</td>
<td>101</td>
</tr>
<tr>
<td>Pets</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Livestock (includes cattle + other non-avian)</td>
<td>5 (1+4)</td>
<td>6 (1+5)</td>
<td>37 (24+13)</td>
<td>39 (25+14)</td>
</tr>
<tr>
<td>Avian (includes wild and domestic)</td>
<td>6</td>
<td>11</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td>Wildlife (non-avian)</td>
<td>29</td>
<td>39</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>80</td>
<td>207</td>
<td>230</td>
</tr>
</tbody>
</table>

**Library size**

- **RCV** - 150 isolates that representing over 2,000 individual fecal samples and approx. 6,000 *E. coli* isolates

- 80 isolates from Lake Granbury fecal
  - Few pet and domestic sewage/septage
  - Fecal samples collected not necessarily most representative of potential pollution sources
  - Could affect identification of those sources

**Identification rate for water isolates**

- 43% of water isolates unidentified
- May only be reduced by including more Lake Granbury fecal isolates in library
**E. coli Source Identifications**

**11861 Lake Granbury at 377**

- **E. coli long-term geo. mean = 5 MPN/100 ml**

![Bar chart showing sources of E. coli](image)

**E. coli Source Identifications**

**18015 Sky Harbor**

- **E. coli long-term geo. mean = 102 MPN/100 ml**

![Bar chart showing sources of E. coli](image)
**E. coli Source Identifications**

**18018 Waters Edge**

- **E. coli long-term geo. mean = 19 MPN/100 ml**

**E. coli Source Identifications**

**20215 Indian Harbor**

- **E. coli long-term geo. mean = 108 MPN/100 ml**
**E. coli Source Identifications**
18038 Port Ridgea East

![Graph showing % of water isolates](image)

- Sewage/Septage: n = 48
- Pets: n = 7
- Livestock: n = 3
- Avian: n = 3
- Wildlife: n = 4
- Unidentified: n = 16

*E. coli* long-term geo. mean = 120 MPN/100 ml

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**Library Independent Screening of Pollution Sources Using Bacteroidales PCR**

*What are Bacteroidales?*

- Human and animal fecal bacteria similar to *E. coli*
- Order Bacteroidales (or class Bacteroidetes) include several different genera and species of bacteria, including *Bacteroides* and *Prevotella* spp.
- Obligate anaerobes – difficult to grow and less likely to multiply in the environment
- More abundant in feces than *E. coli*

*Many different Bacteroidales spp./strains shared between different animals and humans*

*Markers (PCR primers) developed to subgroups of Bacteroidales that appear host specific*
Library Independent Screening of Pollution Sources Using *Bacteroidales* PCR

- Markers available for
  - Ruminants (cattle, deer, goats, sheep, llamas, horses, elk and some non-ruminant wildlife such as some feral hogs)
  - Humans
  - Hogs (including feral hogs)
  - Horses (needs optimization and validation)
  - Birds - under commercial development and needs validation

- No specific markers for wildlife
- Rapid and less expensive than library methods
- Qualitative or semi-quantitative detection
- Relationship to *E. coli* and pathogens uncertain

Sample Processing for *Bacteroidales* PCR

- 100 ml water sample collected, same as for *E. coli*
- Sample filtered to concentrate *Bacteroidales* bacteria
- DNA extraction and purification
- DNA tested for the presence of group-specific *Bacteroidales* – i.e. “markers”
  - standard PCR (presence/absence)
  - qPCR (semi-quantitative)
**Bacteroidales PCR**

Human Marker

Ruminant Marker

**Bacteroidales PCR**

Feral Hog Fecal Marker

Di Giovanni and Casarez, 2007, unpublished
**Bacteroidales PCR Specificity**


<table>
<thead>
<tr>
<th>Source of individual focal samples</th>
<th>No. of samples</th>
<th>No. of samples with indicated result* with the following primers:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General Bac32/</td>
<td>Human HF183/F Bac708R</td>
<td>Human HF183/F Bac708R</td>
</tr>
<tr>
<td>Human</td>
<td>44</td>
<td>34 +, 5 +/−, 1 +/−</td>
<td>41 +, 2 +/−, 1 +/−</td>
</tr>
<tr>
<td>Pig</td>
<td>55</td>
<td>55 +, 4 +/−, 1 +/−</td>
<td>32 +, 4 +/−, 1 +/−</td>
</tr>
<tr>
<td>Cow</td>
<td>32</td>
<td>28 +, 4 +/−, 1 +/−</td>
<td>32 +, 4 +/−, 1 +/−</td>
</tr>
<tr>
<td>Sheep</td>
<td>12</td>
<td>12 +, 1 +/−, 1 +/−</td>
<td>8 +, 2 +/−, 2 +/−</td>
</tr>
<tr>
<td>Chicken</td>
<td>10</td>
<td>4 +, 3 +/−, 6 +/−</td>
<td>7 +, 7 +/−, 7 +/−</td>
</tr>
<tr>
<td>Wild bird</td>
<td>45</td>
<td>7 +, 7 +/−, 7 +/−</td>
<td>7 +, 7 +/−, 7 +/−</td>
</tr>
</tbody>
</table>

* +, positive signal; −, negative signal; +/−, weak positive result or both positive and negative results were obtained in three replicate analyses.

**Bacteroidales PCR Specificity**

Vogel et al., J Environ Qual (2007)

- 15 Septic, 100 cattle, 6 horse, 62 wildlife (mostly raccoons and opossums) samples
- General (Bac32/708) marker: >90% in human, cattle, horse; only 32% in wildlife
- Ruminant (CF128/Bac708): >90% in cattle and horse; <3% in human and wildlife
- Human (HF183/BAC708): 40% in human; 1% cattle, 0% horse, 0% wildlife
### Presence/absence Detection of Bacteroidales Markers

<table>
<thead>
<tr>
<th>Site</th>
<th>Name</th>
<th># samples</th>
<th>Universal %</th>
<th>Hog %</th>
<th>Human %</th>
<th>Ruminant %</th>
</tr>
</thead>
<tbody>
<tr>
<td>11061</td>
<td>Main Lake</td>
<td>6</td>
<td>100</td>
<td>33</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>18015</td>
<td>Sky Harbor</td>
<td>6</td>
<td>100</td>
<td>67</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>18016</td>
<td>Sky Harbor Field Duplicate</td>
<td>6</td>
<td>100</td>
<td>67</td>
<td>67</td>
<td>50</td>
</tr>
<tr>
<td>19016</td>
<td>Waters Edge</td>
<td>6</td>
<td>100</td>
<td>50</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>20215</td>
<td>Indian Harbor</td>
<td>6</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>18038</td>
<td>Port Ridgelea E</td>
<td>6</td>
<td>100</td>
<td>67</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

*Ruminant marker detects deer, cattle, llamas, goats and some other non-ruminant wildlife, including feral hogs.*

### Bacteroidales PCR Specificity

**Lake Granbury Fecal Samples**

- Some sewage samples weakly positive for hog marker
- Several wildlife samples positive for human marker
  - Deer atypical
  - Coyotes and rabbits not tested often in other studies!
**Bacteroidales qPCR**

![Graph showing qPCR data]

**Bacteroidales qPCR Considerations**

- Can use qPCR for evaluating relative abundance of marker *in individual samples*
  - General *Bacteroidales* marker baseline
  - Relative abundance of other markers
  - May provide more detailed information than presence/absence data—especially for individual sampling location

- However, although markers can be *detected* quantitatively, quantitative estimates of fecal loading may not follow
  - Differences in fecal abundance, environmental persistence and PCR sensitivity for different *Bacteroidales* markers
Bacteroidales qPCR Analytical Precision

* Analytical precision
  - *Bacteroidales* human marker occurrence in duplicate analysis of each water sample DNA extract for Sky Harbor duplicates (18015 and 18015FD, 12 individual samples)
  - Average threshold cycle (C\text{\textsubscript{T}}) of samples = 36.96
  - Average standard deviation of replicate C\text{\textsubscript{T}} values = 1.5
  - Average Relative Standard Deviation of replicates = 4.3% (range 0.2 to 10.3%)
  - Results in an average error of approximately 2-fold difference in marker quantitation

Therefore, when comparing one sample to another the difference must be greater than 2-fold to be significant. Results can sometimes vary between replicate samples. *Looking for trends.*

Bacteroidales qPCR

Human Marker Occurrence by Station and Sample Batch

![Graph showing human marker abundance by station and sample batch](image)
BST Identification of Likely Pollution Sources

- 11861 Lake Granbury at 377 – wildlife (deer, feral hogs); sewage/septage; livestock
- 18015 Sky Harbor – wildlife (deer, feral hogs); sewage/septage
- 18018 Waters Edge – NPS; wildlife (feral hogs)
- 20215 Indian Harbor – wildlife (deer, feral hogs); livestock
- 18038 Port Ridglea East – wildlife (deer, hogs); livestock; conflicting results for sewage/septage

Discussion

- Sewage/septage E. coli and/or Bacteroidales markers found at all sites, but does not appear to be leading pollution source
- Feral hogs identified as a significant source
- Unexpected feral hog source at Waters Edge
  - Two “hot” samples – impact from main lake water?
  - Additional samples could resolve issue
  - Low E. coli geo. mean of 19 MPN/100 ml
For Further Information

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