



Bacterial Source Tracking

Little Brazos River Tributaries Bacteria Assessment Project

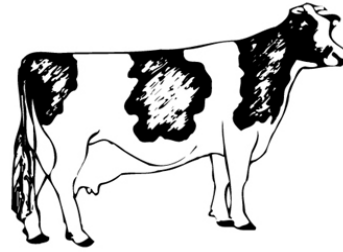
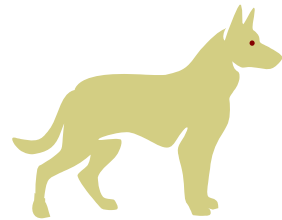
Terry Gentry

*Soil & Aquatic Microbiology Laboratory
Department of Soil & Crop Sciences
Texas A&M University*

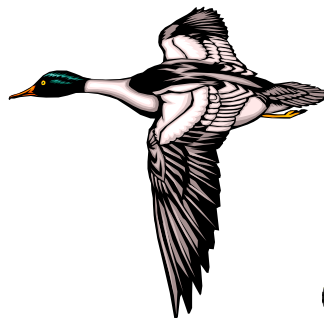
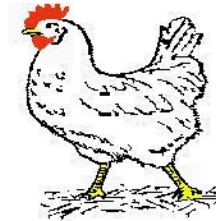
October 14, 2008

Improving Life through Science and
Technology.

Sources of Bacteria (*E. coli*)



▪ Sources of bacteria





What is BST?

Bacterial Source Tracking

- **Data collection and analysis to determine the sources of fecal contamination in a waterbody**
- **Based on uniqueness of bacteria from individual sources – a variety of different methods are used**
- **Differs from modeling in that it is not a predictive tool and does not require calibration and validation of input variables**



BST Methods

- A variety of different methods have been used
- Can be classified according to approach:
 - Phenotypic v. Genotypic
 - Library-dependent v. Library-independent

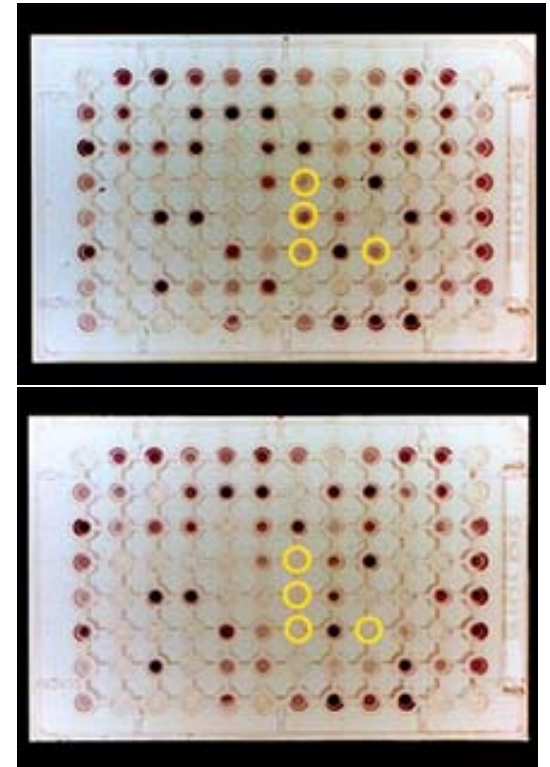
Phenotypic BST Methods

Methods:

- Kirby-Bauer Antibiotic resistance analysis (ARA)
- Carbon source utilization (CSU)

Advantages/Disadvantages:

- Less expensive
- Less discriminating





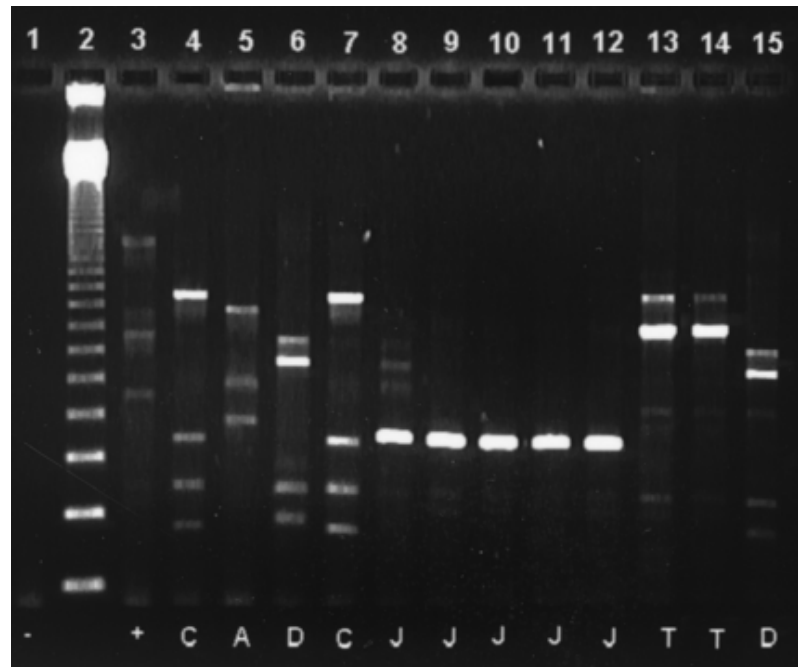
Genotypic BST Methods

Methods:

- **DNA fingerprinting**
 - **Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)**
 - **Ribotyping or RiboPrinting® (RP)**
 - **Pulsed-field gel electrophoresis (PFGE)**

Genotypic BST Methods

Methods:



Advantages/Disadvantages:

- More expensive
- More discriminating



Library-Dependent BST

Approach:

- Isolate *E. coli* from known sources
- Characterize isolates using phenotypic or genotypic techniques (e.g., ERIC-PCR, RP)
- Match unknown environmental isolates (water samples) against known-source library

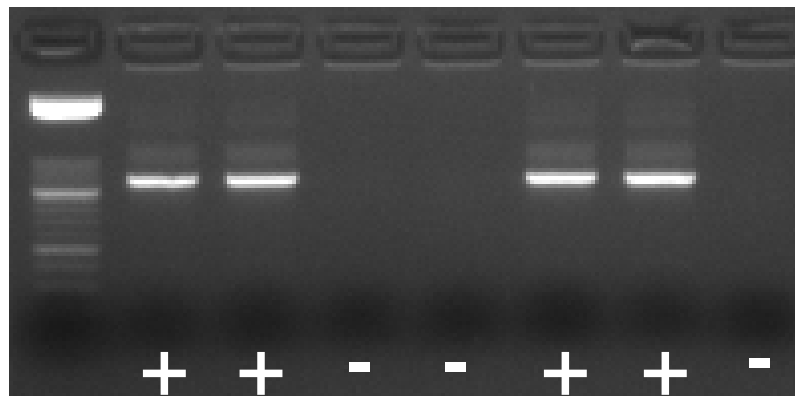
Considerations:

- Library construction expensive
- Regional effects on library?

Library-Independent BST

Approach:

- Genotypic detection of microorganisms based on marker genes
- Does not require known-source library
- Most common approach targets *Bacteroidales*





What are *Bacteroidales*?

- More abundant in feces than *E. coli*
- Obligate anaerobes – less likely to multiply in environment
- Subgroups appear to be host specific
- Markers available for humans, ruminants, horse, swine
- Not pathogens



Library-Independent BST

Considerations:

- **Rapid and less expensive than library-dependent methods**
- **Limited markers – human, ruminant, horse, swine for *Bacteroidales***
- **New markers being developed**



Which BST Methods Should be Used in Texas?

- **Bacteria TMDL Task Force evaluated numerous methods**

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Direct comparison of four bacterial source tracking methods and use of composite data sets

E.A. Casarez¹, S.D. Pillai², J.B. Mott³, M. Vargas⁴, K.E. Dean⁴ and G.D. Di Giovanni¹

¹ Texas Agricultural Experiment Station, Texas A&M University Agricultural Research Center, El Paso, TX, USA

² Department of Poultry Science, Texas A&M University, College Station, TX, USA

³ Department of Physical and Life Sciences, Texas A&M University-Corpus Christi, TX, USA

⁴ Parsons Water and Infrastructure, Inc., Austin, TX, USA

Keywords

antibiotic resistance, enterobacterial repetitive intergenic consensus polymerase chain reaction, *Escherichia coli*, pulsed-field gel electrophoresis, ribotyping, water quality faecal pollution.

Correspondence

George D. Di Giovanni, Texas Agricultural Experiment Station, Texas A&M University

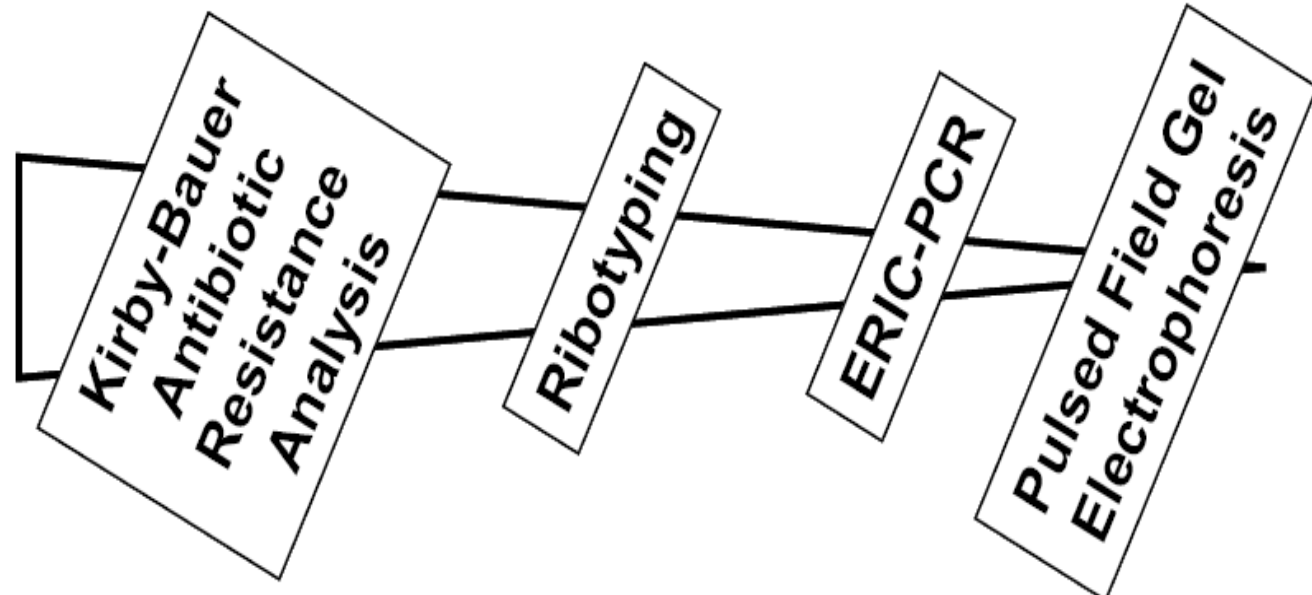
Abstract

Aims: Four bacterial source tracking (BST) methods, enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), automated ribotyping using *Hind*III, Kirby-Bauer antibiotic resistance analysis (KB-ARA) and pulsed-field gel electrophoresis (PFGE) were directly compared using the same collection of *Escherichia coli* isolates. The data sets from each BST method and from composite methods were compared for library accuracy and their ability to identify water isolates.

BST Methods Evaluated

Lowest
Resolution

Highest
Resolution





Which BST Methods Should be Used in Texas?

Recommended Methods:

- **Library-dependent**
 - **Combination of methods (ERIC-RP, ERIC-ARA, or CSU-ARA)**
- **Library-independent**
 - ***Bacteroidales* PCR for human, ruminant, horse, and swine sources**

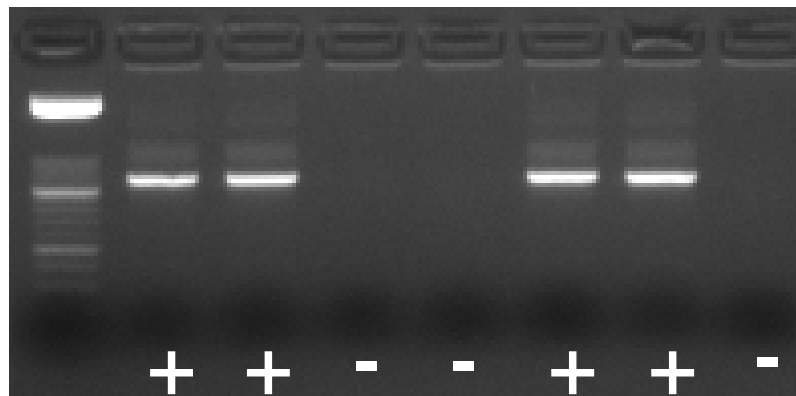


BST for Little Brazos River Tributaries

- **Tier 2 Analysis (Bacteria TMDL Task Force Report)**
 - Targeted water quality monitoring
 - Land use analysis and modeling
 - Bacterial source tracking
 - Library-independent BST
 - Limited library-dependent BST
 - Analyze data with stakeholders

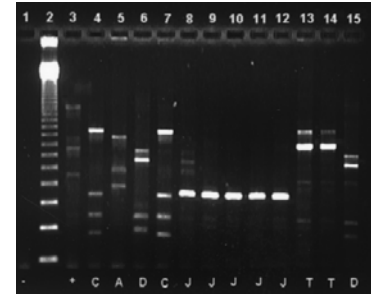
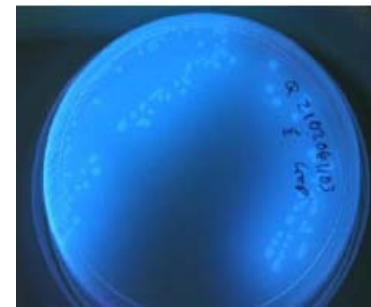
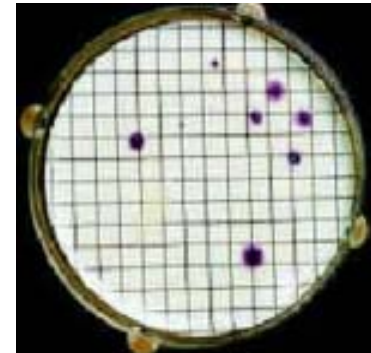
BST for Little Brazos River Tributaries

- **Library independent**
 - Analyze 50-100 water samples per segment (~250 total samples)
 - *Bacteroidales* PCR for human, ruminant, horse, and swine markers



BST for Little Brazos River Tributaries

- **Limited library- dependent**
 - Analyze *E. coli* from 50-100 water samples from across the entire study area
 - Confirmation as *E. coli* using biochemical tests
 - ERIC-RP fingerprinting





Next Steps for BST

- **A sanitary survey design meeting will be held – outcomes will help AgriLife Research understand usefulness of existing known source library for BST**
- **Brazos River Authority will begin collecting water samples and AgriLife Research will begin BST on a subset of those samples**
- **AgriLife Research will be back at a stakeholder meeting in ~May 2009 to provide an update on the progress of BST**
- **BST should be completed ~February 2010**



Questions?

Terry Gentry

2474 TAMU

Texas A&M University

College Station, TX 77843

Phone: (979) 845-5323

Email: tgentry@ag.tamu.edu