



# ***E. coli* Bacterial Source Tracking in Texas**

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# Acknowledgments

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## Co-Principal Investigators and Staff

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**Anthony Sisk**

**Adriana Galindo**

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## Collaborators

**Parsons**

**James Miertschin and Associates**

**Texas Farm Bureau**

**City of Waco**

**Brazos River Authority**

## Funding

**Texas Commission on Environmental Quality (TCEQ)**

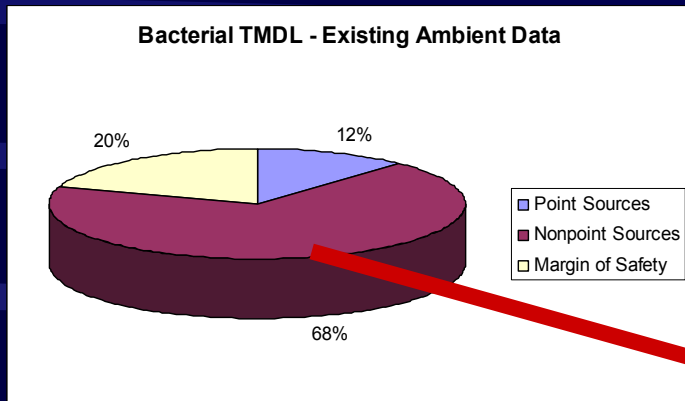
**Texas State Soil and Water Conservation Board (TSSWCB)**

**Environmental Protection Agency**

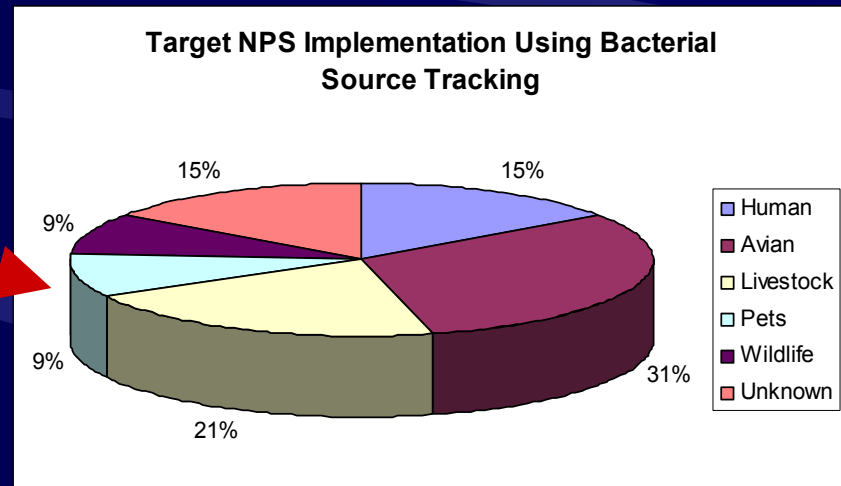


# TMDLs and BST

Total maximum daily load (TMDL) is the calculated maximum amount of pollution that a water body can receive and still meet water quality standards – developed for fecal bacteria, e.g. *E. coli*, and other contaminants



Typical bacterial TMDL – gross allocations into point sources and nonpoint sources based on land use



BST used to better define nonpoint sources of pollution and aid development of effective bacterial TMDLs



# What the Heck is *E. coli*?

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*Escherichia coli* (*E. coli*) is a bacterium naturally found in the intestines and the **feces** of warm-blooded animals

Many different types, most harmless, but some may cause illness (e.g. hamburger restaurant outbreaks)

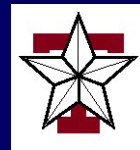
Laboratory tests can distinguish one strain from another

Commonly used as an indicator of fecal pollution of water

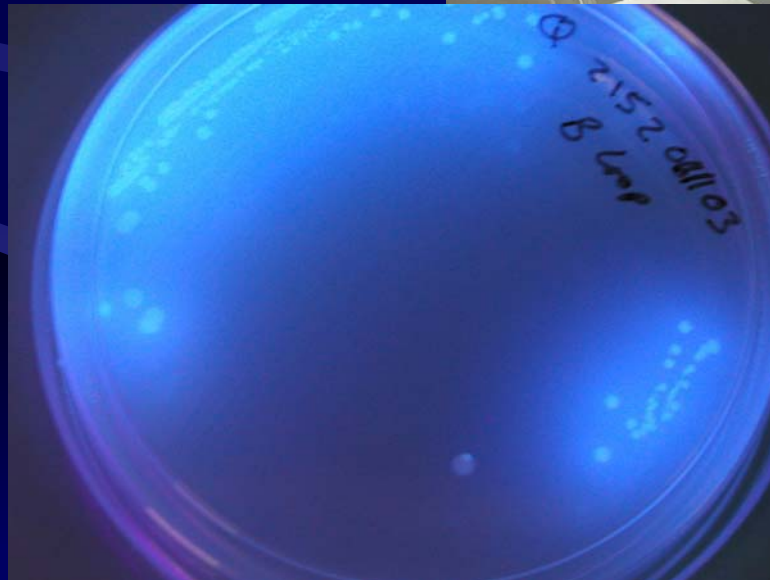
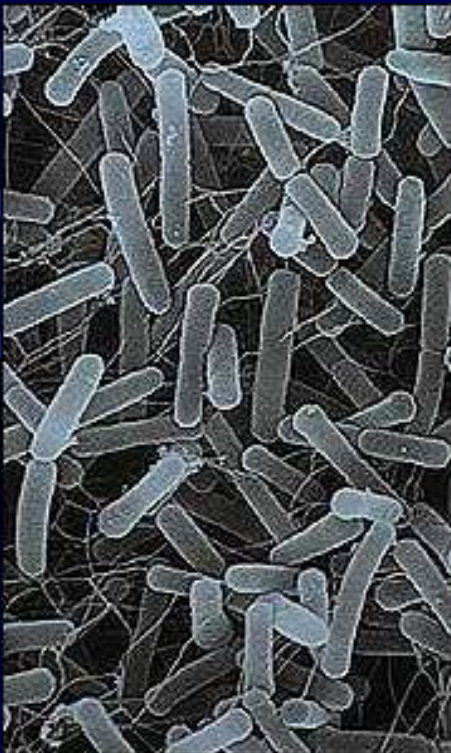
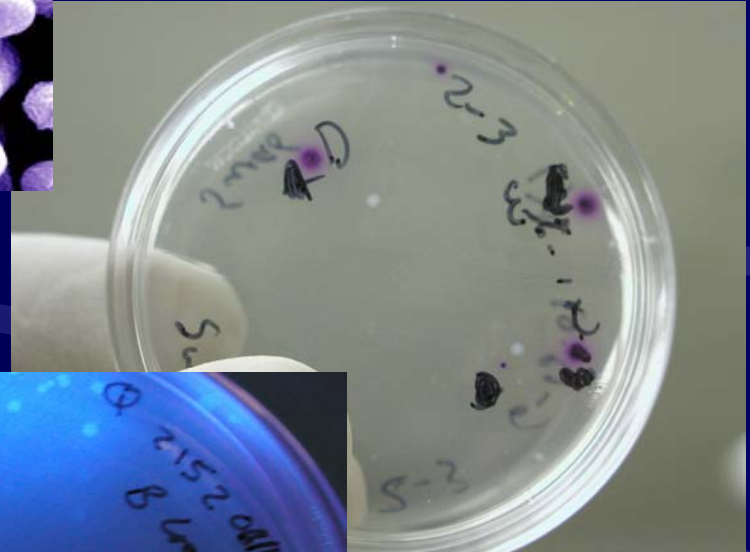
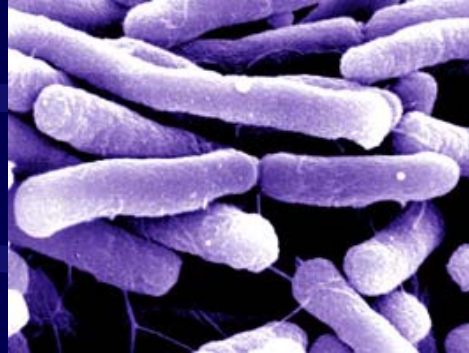
## Texas Water Quality Criteria

*E. coli* for contact recreation

- 🔥 long-term geometric mean  $\leq 126/100$  mL
- 🔥 single sample  $\leq 394/100$  mL



# What Does *E. coli* Look Like?



# There's *E. coli* in the Water, But Where Did it Come From?

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- 💧 Bacterial Source Tracking (BST) uses 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 to identify sources of water isolates
- 💧 “Local” watershed libraries of known source *E. coli* isolates currently considered most useful for identifying *E. coli* obtained from ambient water samples



# Current BST Studies

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## Texas Commission on Environmental Quality (TCEQ)

- 💧 San Antonio River, Salado and Peach Creeks
- 💧 Over 3,000 *E. coli* isolated from 750 known source samples and over 3,000 *E. coli* isolated from 850 ambient water samples
- 💧 BST analyses underway

## Texas State Soil and Water Conservation Board (TSSWCB)

- 💧 North Bosque and Leon River Watersheds – Lakes Waco and Belton
- 💧 Sampling campaign Sept. 2003 – Aug. 2004



# Objectives

## Bacterial Source Tracking Projects

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- Develop publicly available, genetic and phenotypic fingerprint libraries of *E. coli* isolates from known animal and human sources
- Determine the reproducibility and accuracy of the methods
- Determine the potential human or animal sources of *E. coli* contamination of water to aid development of TMDLs and watershed protection strategies
- Provide a foundation for additional watershed BST studies





# Ability of Methods to Discriminate Differences Between Bacterial Strains Bacterial Source Tracking Projects

Lowest  
Discrimination

Highest  
Discrimination



Which method or combination is best?

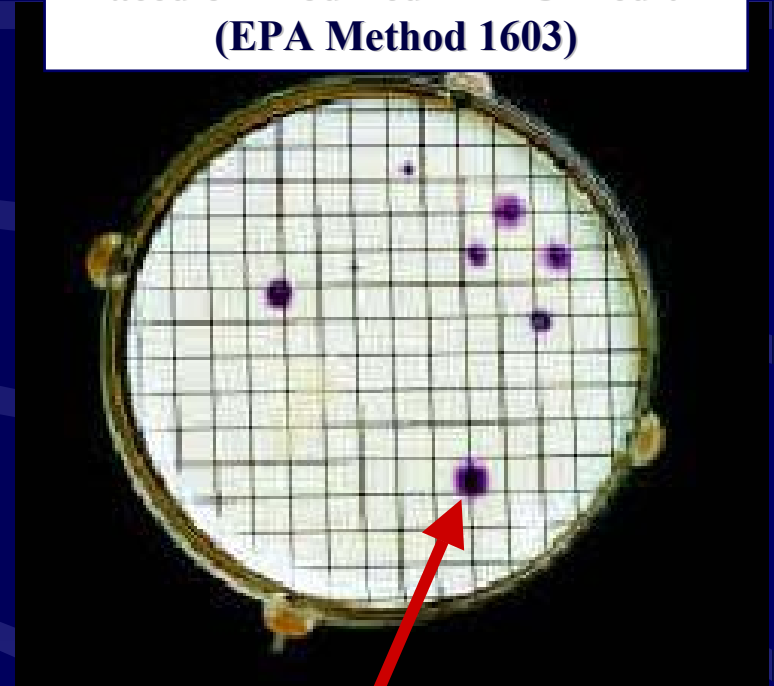


# Isolation of *E. coli* From Feces and Water

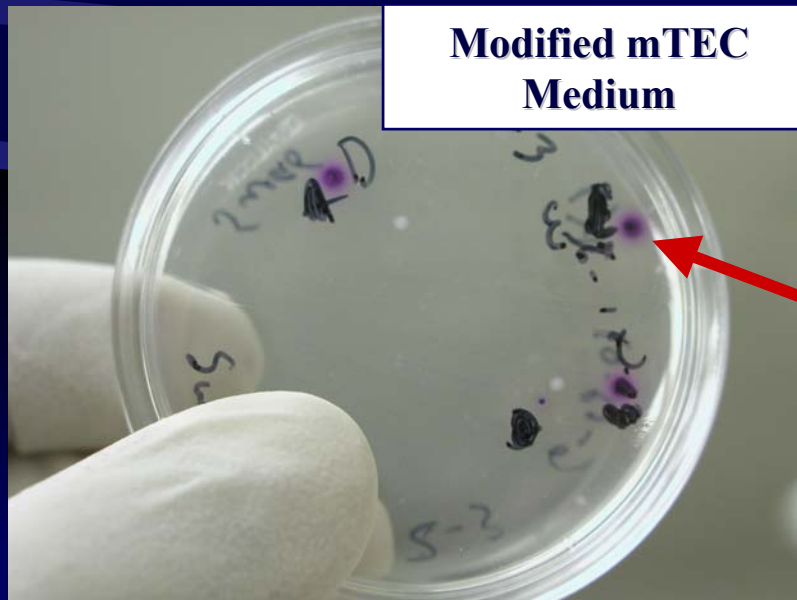
**Fecal Specimens**



**Water Sample Filtered and Filter Placed on Modified mTEC Medium  
(EPA Method 1603)**



**Modified mTEC Medium**



***E. coli* Colonies**

Each *E. coli* colony is called an "Isolate"



# Purification and Confirmation of *E. coli*



# Archival (Freezing) of *E. coli* Isolates

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Isolates stored  
frozen at  $-80\text{ }^{\circ}\text{C}$   
( $-112\text{ }^{\circ}\text{F}$ )

Remain alive for  
years

Living library of  
isolates can be  
shared with  
other  
researchers



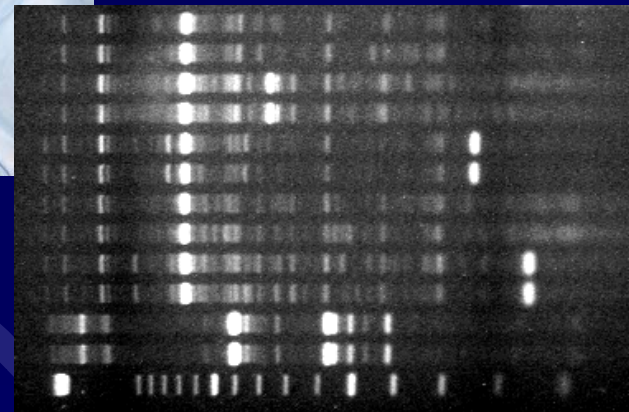
# BST Technique 1

## 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

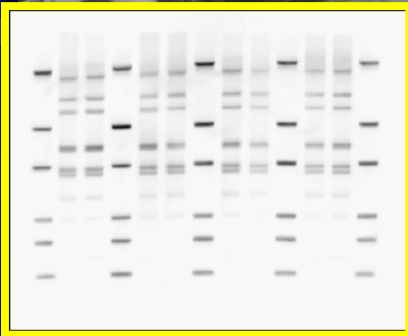


# BST Technique 2

## Automated Ribotyping



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



Sample Number	Label	RiboGroup	Similarity to Selected 295-21-S-1	RiboPrint® Pattern			
				1 kbp	5	10	15 50
295-21-S-1	QC 101	HindIII 295-21-S-1	1.00				
295-21-S-2	QC 101	HindIII 295-21-S-1	0.98				
295-21-S-3	QC 101	HindIII 295-19-S-1	0.95				
295-21-S-4	QC 101	HindIII 295-21-S-1	0.97				
295-21-S-5	QC 101	HindIII 295-21-S-1	0.97				
295-21-S-6	QC 101	HindIII 295-21-S-1	0.96				
295-21-S-7	QC 101	HindIII 295-21-S-1	0.93				
295-21-S-8	QC 101	HindIII 295-21-S-1	0.94				



# BST Technique 3

## Pulsed Field Gel Electrophoresis (PFGE)

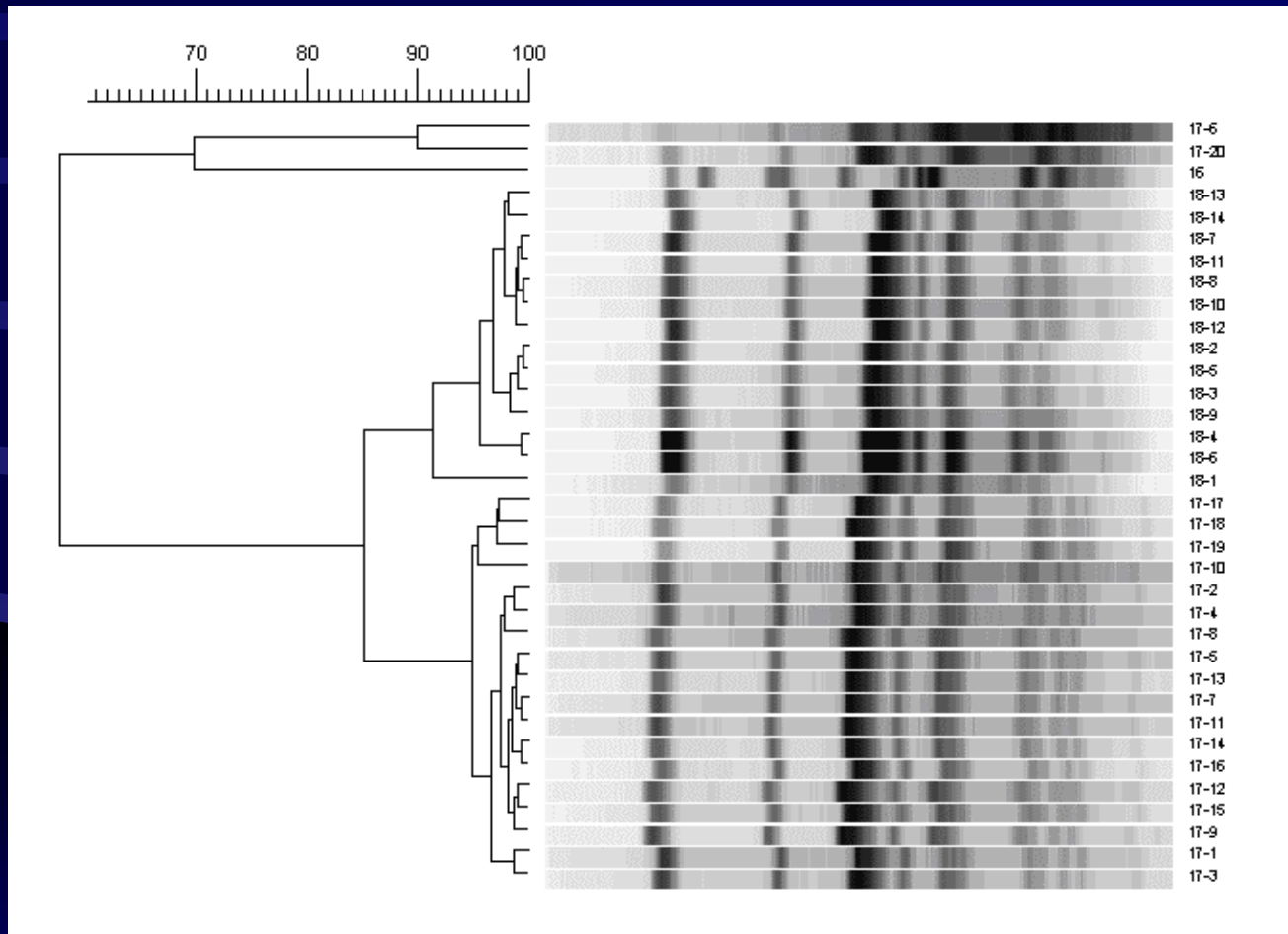


Photo Courtesy of Dr. S. D. Pillai



# BST Technique 4

## Antibiotic Resistance Analysis (ARA)

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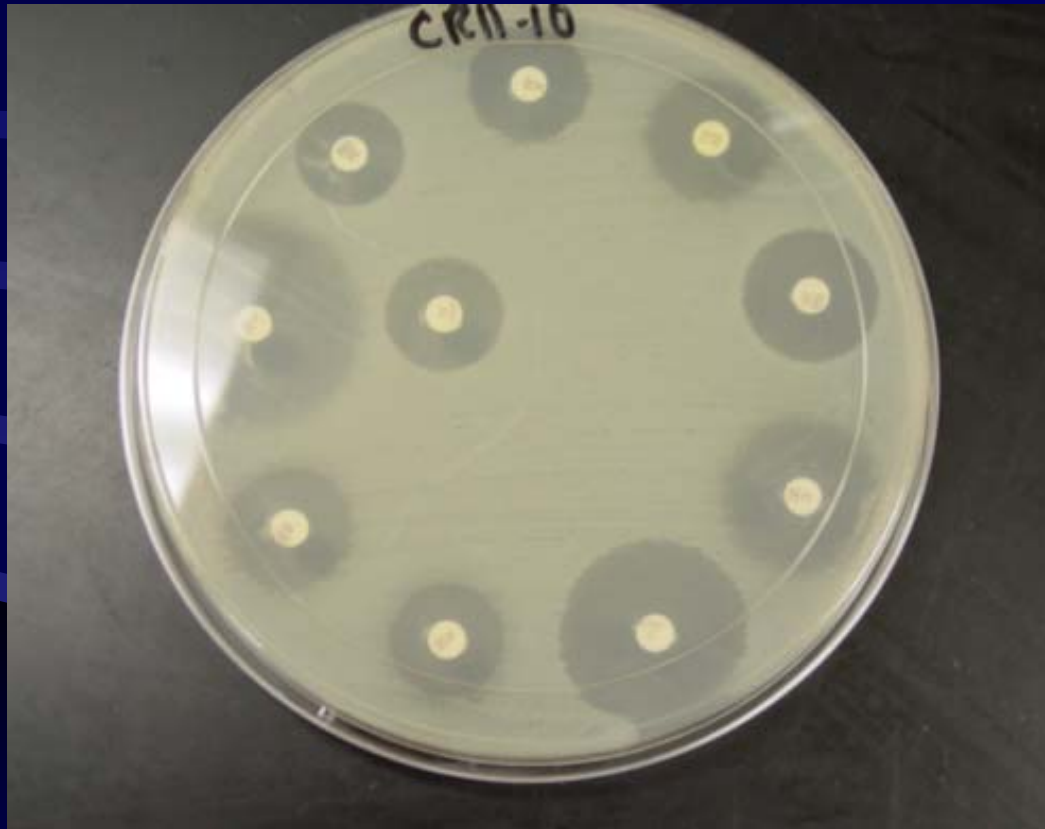


Photo Courtesy of Dr. J. Mott





# Data Analysis

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**DNA Fingerprints and antibiotic resistance patterns of isolates from water samples compared to *E. coli* reference library to identify likely human or animal sources of pollution**

**Applied Maths BioNumerics software**

- ◆ **Used by CDC for PulseNet**
- ◆ **Gaining popularity in the BST field**
- ◆ **Performs multiple statistical tests of each data set individually and in combination – Discriminant analysis, ARCC, dendrogram significance tools, congruence between data sets**

**Developed *E. coli* libraries will provide a foundation for other BST studies in Texas**



# *For Further Information*

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