Environmental Analysis of Local Recreational Waters for the Presence of *Escherichia coli O157:H7* and other Coliform Bacteria

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Introduction

Recreational Waters

- **Recreational Waters:**
  - body of water used for recreation (swimming, soaking, boating, etc.)
  - structure that encloses this water (CDC)
- **Types of Recreational Waters:**
  - treated or disinfected
  - untreated or naturally occurring

Examples of Recreational Waters

- **Treated or disinfected:**
  - swimming pools
  - water parks
  - spas
  - hot tubs

- **Untreated or naturally occurring:**
  - streams, rivers
  - creeks
  - springs
  - ponds, lakes
  - beaches, oceans

http://www.mediapropictures.com/img/locations/lakes_rivers/bucegi%20lacul%20bolboci.jpg

http://www.friendly-bungalows-tanna-vanuatu.com/images/freddy-on-beach.jpg

http://www.southtravels.com/america/usa/hyattregencyaustin/gifs/pool.jpg

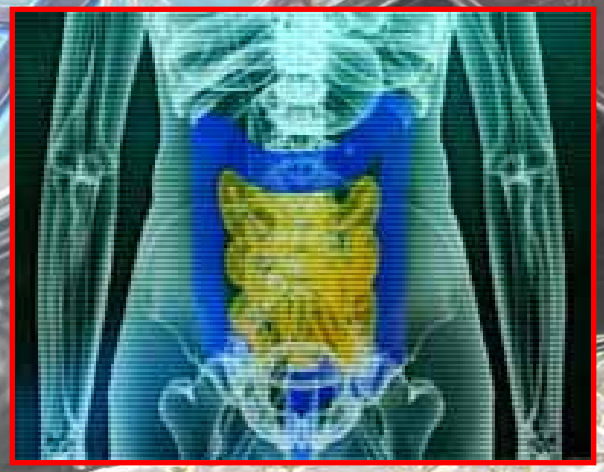
http://www.hot-tub-direct.co.uk/newImages/showers/spa1.jpg

Recreational Water Illnesses

- Recreational Water Illnesses (RWIs)
  - Any illness caused by exposure to contaminated water
    - accidental defecation
    - sewage
    - wounds from diseased individuals

Manifestations of RWIs

- **Gastroenteritis (most common)**
- **Dermatitis**
- **Meningoencephalitis**
- **Folliculitis**
- Asymptomatic excretion
- Abdominal cramps
- Acute respiratory infections
- Hemorrhagic colitis
- Death

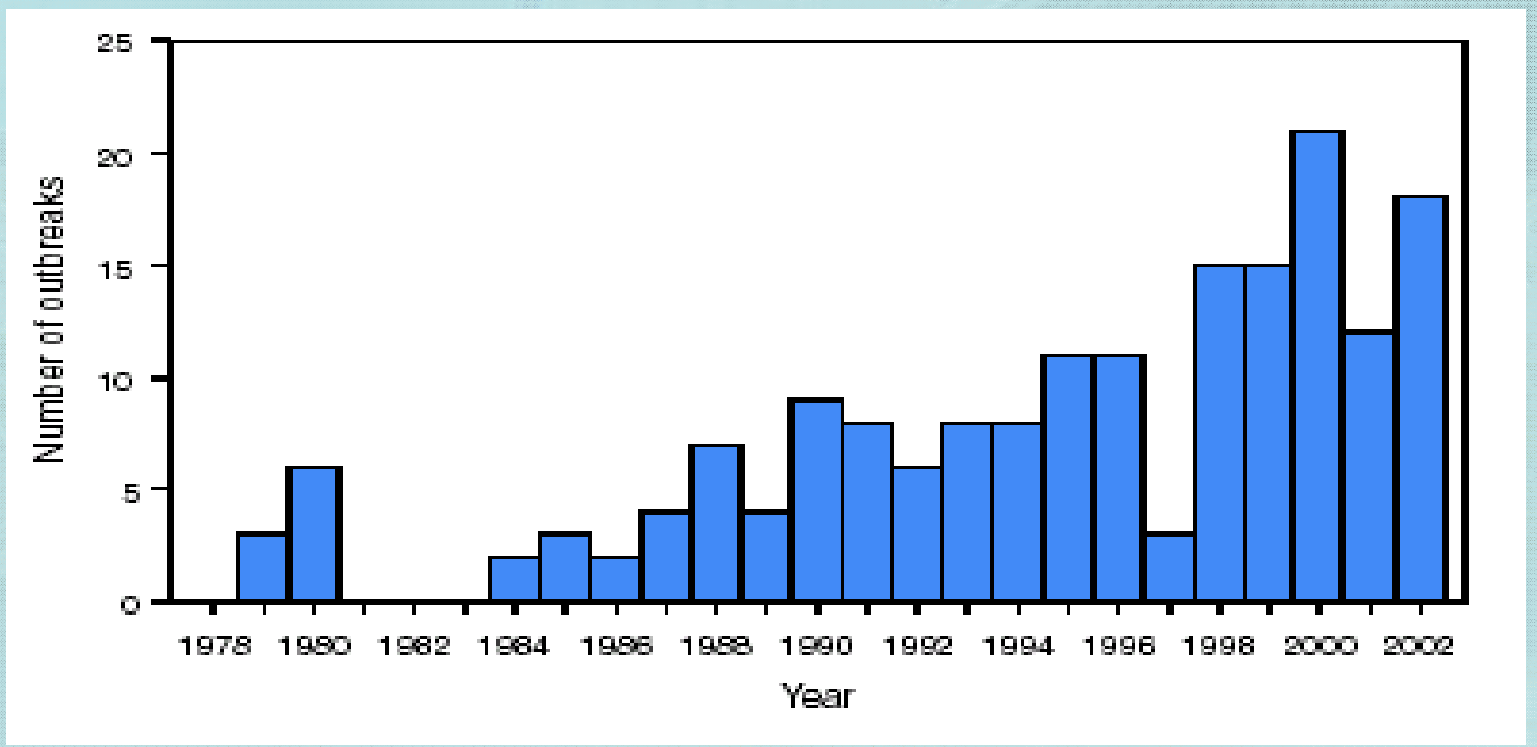


http://motrildigital.blogia.com/upload/2004-07-14_gastroenteritis.jpg

http://www.dermatitisherpetiformis.org.uk/library/legs1.jpg

Waterborne-Disease Outbreaks of Gastroenteritis Associated with Recreational Water

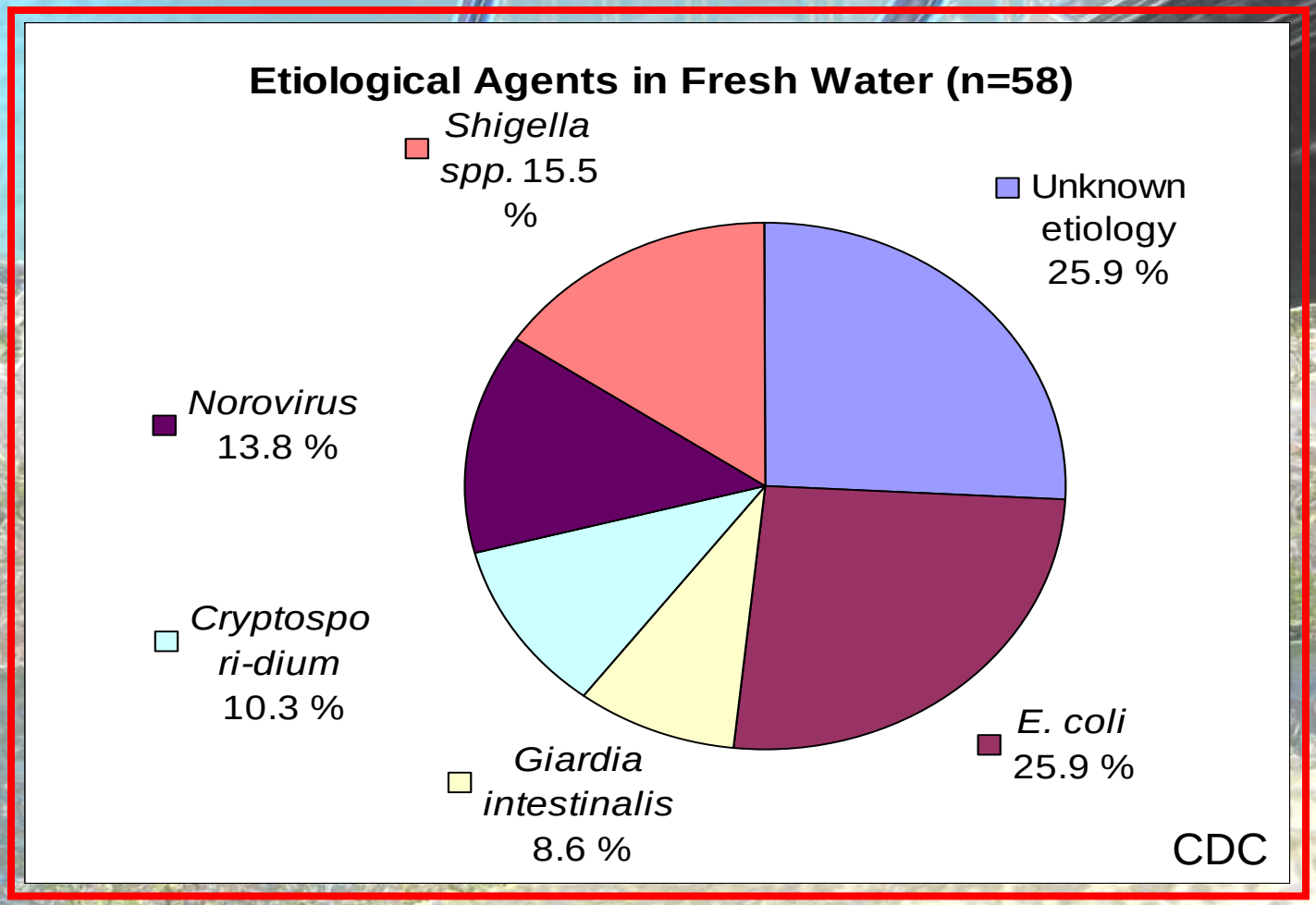
Number of waterborne-disease outbreaks of gastroenteritis (n=176) associated with recreational water, by year – United States, 1978-2002



Facts and Figures

- **2001 to 2002: 65 outbreaks affected 2,536 people nationwide.**
- **Organisms found in ALL recreational waters associated with gastroenteritis:**
  - *Cryptosporidium* species (36.7%)
  - *Norovirus* (16.7%)
  - *Escherichia coli* (13.3%)
  - *Shigella sonnei* (6.7%)
  - *Giardia intestinalis* (3.3%)
  - Unknown etiology (AGI) (23.3%) (CDC)

Distribution of Etiological Agents in Untreated Recreational Waters Associated with Gastroenteritis



Monitoring Recreational Waters

1. **Total Coliforms**
   - aerobic or facultative anaerobic
   - Gram-negative
   - non-spore-forming
   - rod-shaped
   - ferment lactose

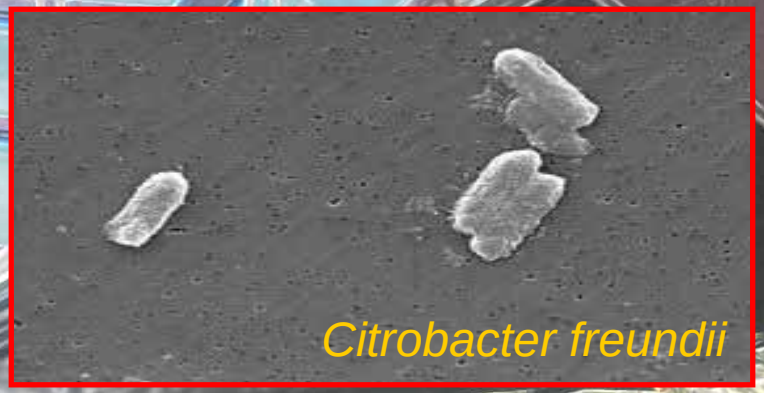
2. **Fecal Coliforms**
   - aid in the digestion of food
   - grow at elevated temperatures
   - associated with fecal material

Standards for the State of Texas

- No standard for the acceptable level of coliforms for fresh water spots.
- Regulation only occurs if a severe outbreak is reported.
- Early detection of harmful bacteria is beneficial.
  - outbreak prevention
  - public health

Coliforms are Members of the Family *Enterobacteriaceae*

- **Family:**
  - *Enterobacteriaceae*
    - **Genera:**
      - **Frank pathogens**
      - *Shigella*
      - *Salmonella*
      - *Yersinia*
      - *E. coli O157:H7*
      - **Opportunists**
      - *Enterobacter*
      - *Citrobacter*
      - *Escherichia*
      - *Klebsiella*

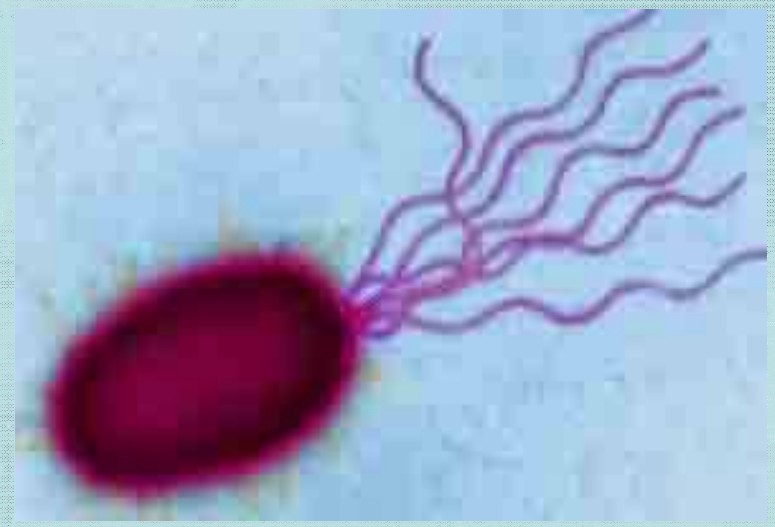


http://upload.wikimedia.org/wikipedia/commons/thumb/5/52/Citrobacter_freundii.jpg/280px-Citrobacter_freundii.jpg

http://www.microscopyconsulting.com/Gallery/images/Shigella%20flexnarii.jpg

Escherichia coli

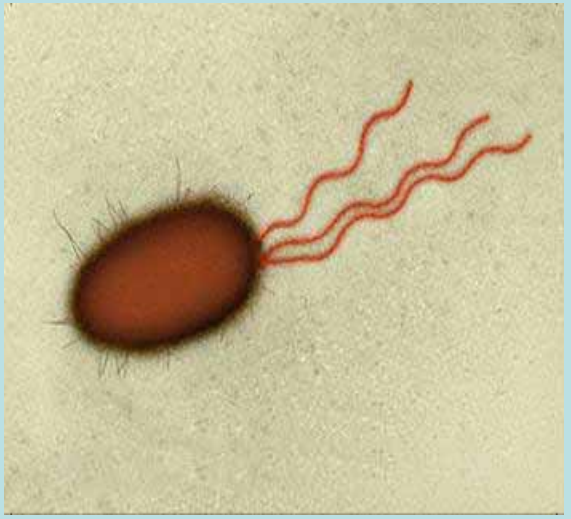
- Discovered by Dr. Theodore von Escherich in 1885.
- Gram negative bacteria that are usually found in the gut of healthy persons and other animals.



- Most are non-pathogenic to man; however, some strains can cause diarrhea and other diseases.

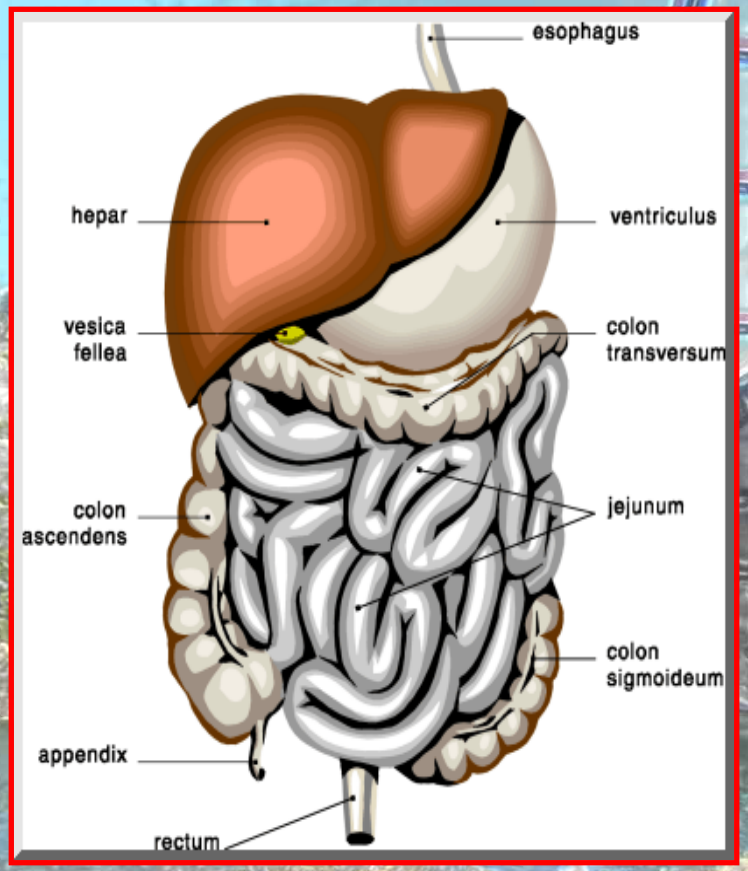
Escherichia coli O157: H7

- Emerging pathogen
- Affects 73,000 people and kills about 61 per year in the US.



- Transmitted by contaminated water, food, and recently recreational water.

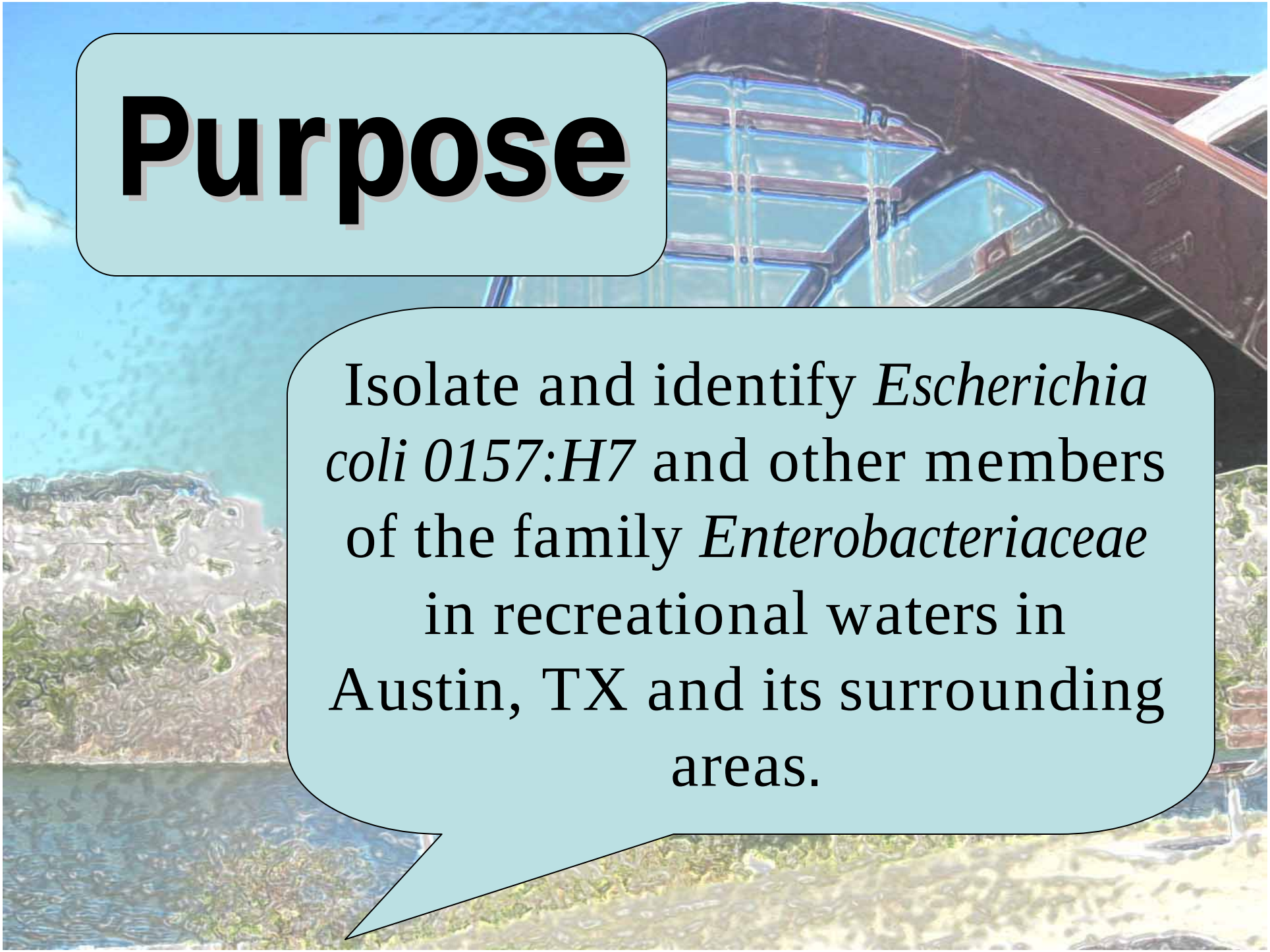
Symptoms of Escherichia coli O157: H7 Disease



http://ww2.saturn.stpaul.k12.mn.us/Kids/HumanBody/Humanbody/dig.gif

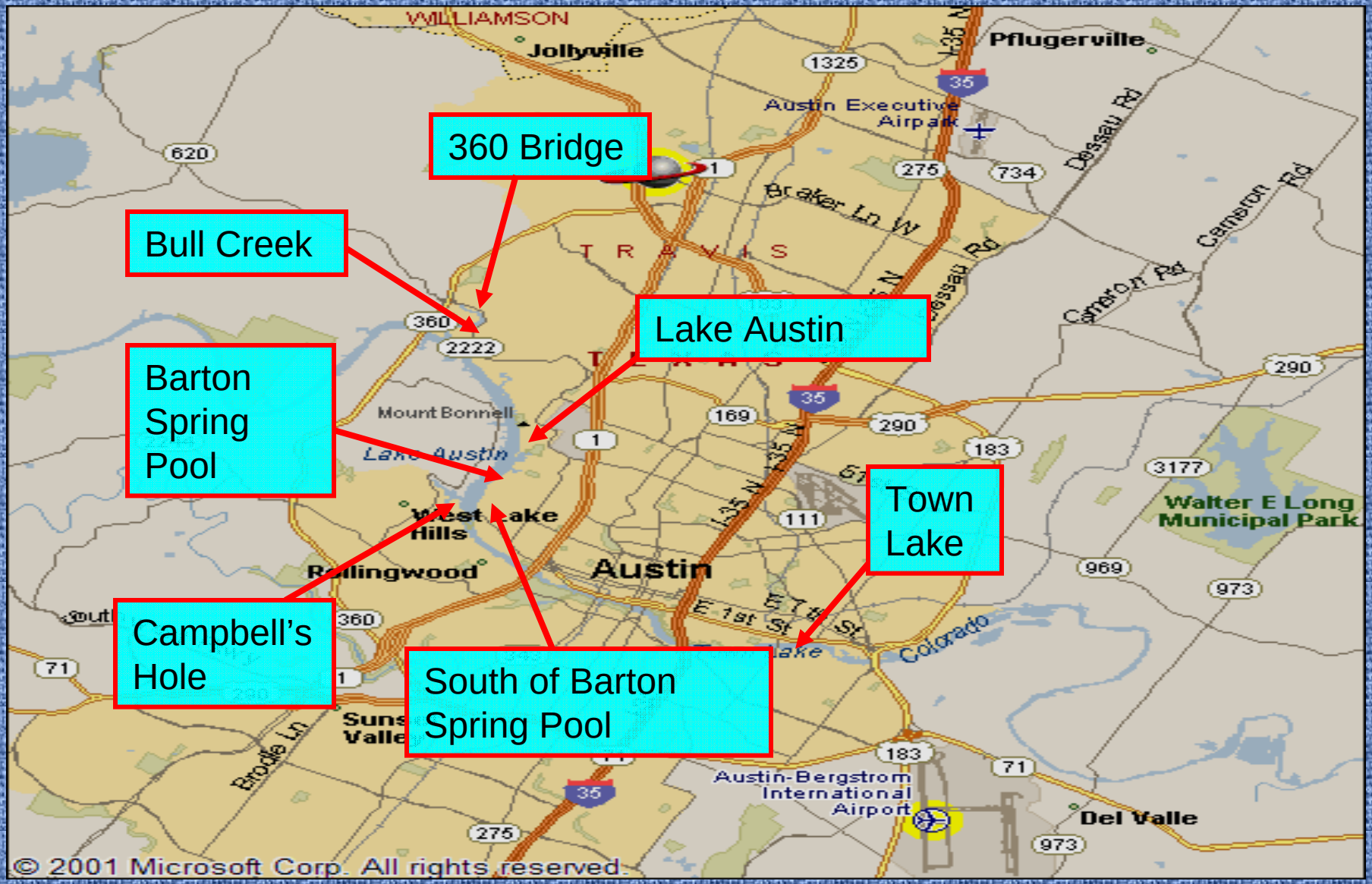
- Symptoms include:
  - Watery diarrhea
  - Bloody diarrhea
  - Abdominal pain
  - Hemorrhagic colitis

  Complication:
  - Hemolytic Uremic Syndrome (HUS)


Purpose
Isolate and identify *Escherichia coli 0157:H7* and other members of the family *Enterobacteriaceae* in recreational waters in Austin, TX and its surrounding areas.


Methods

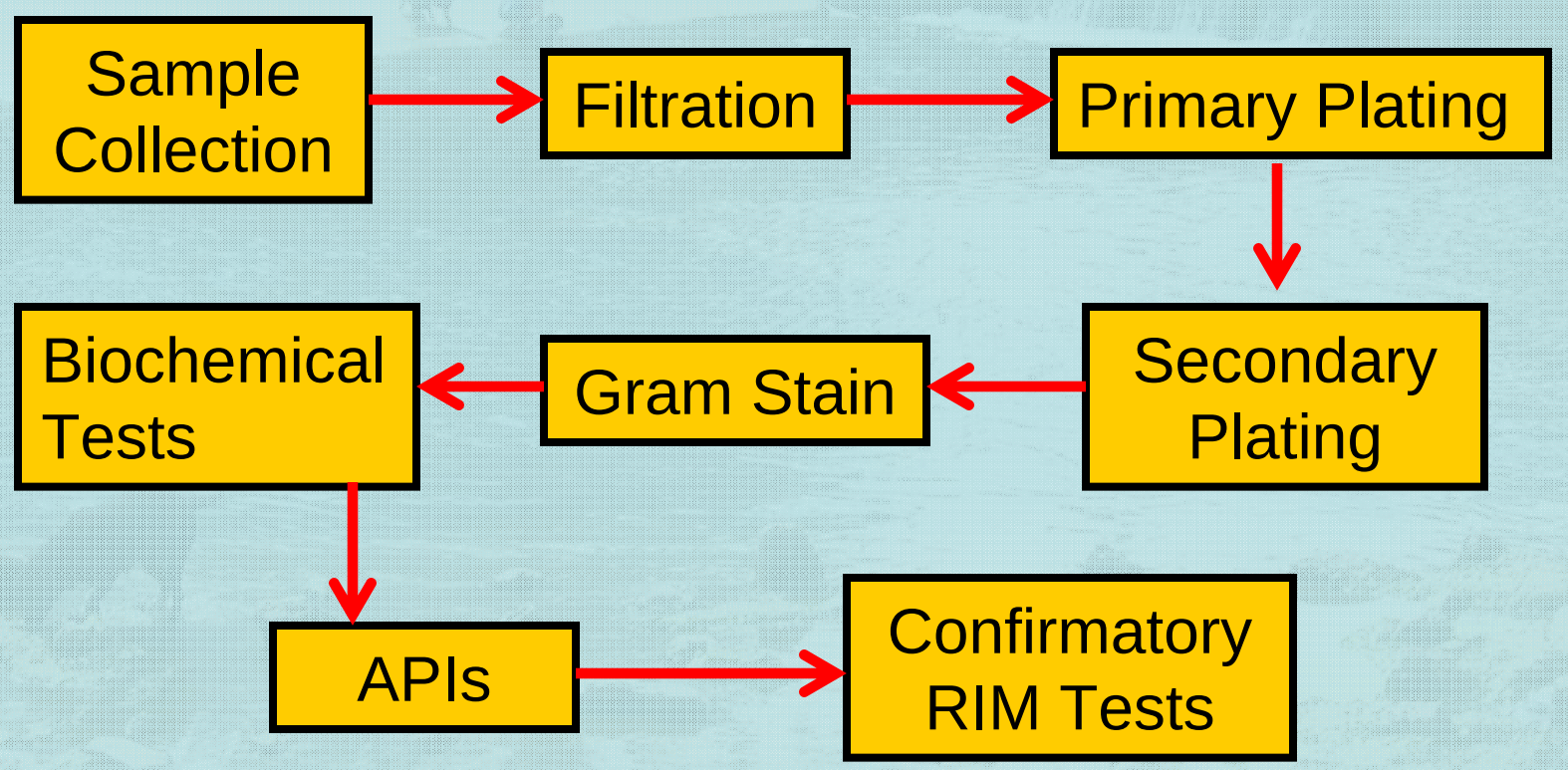
Recreational Water Sources



Study Design

- 7 sites
- Samples were collected weekly
- 188 water samples collected and analyzed

Protocol for Isolation and Identification of Members of the Family *Enterobacteriaceae*

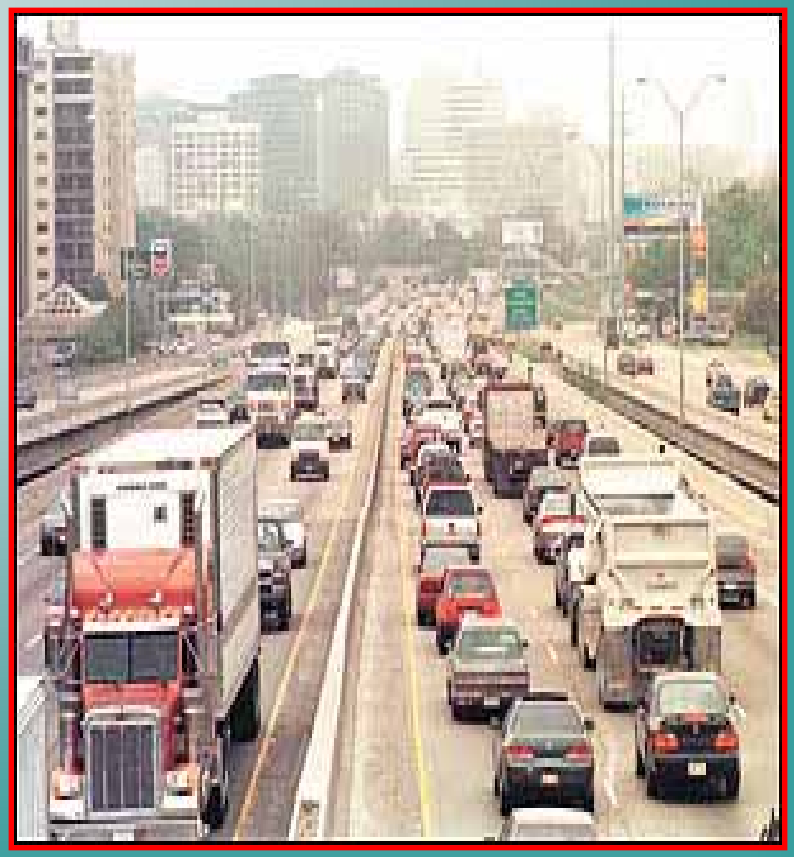


Sample Collection

Samples were collected with sterile collection cups using standard protocol.

Transportation of Samples

Samples were transported to the Consumer Microbiology Laboratory at the TDSHS.



Sample Filtration

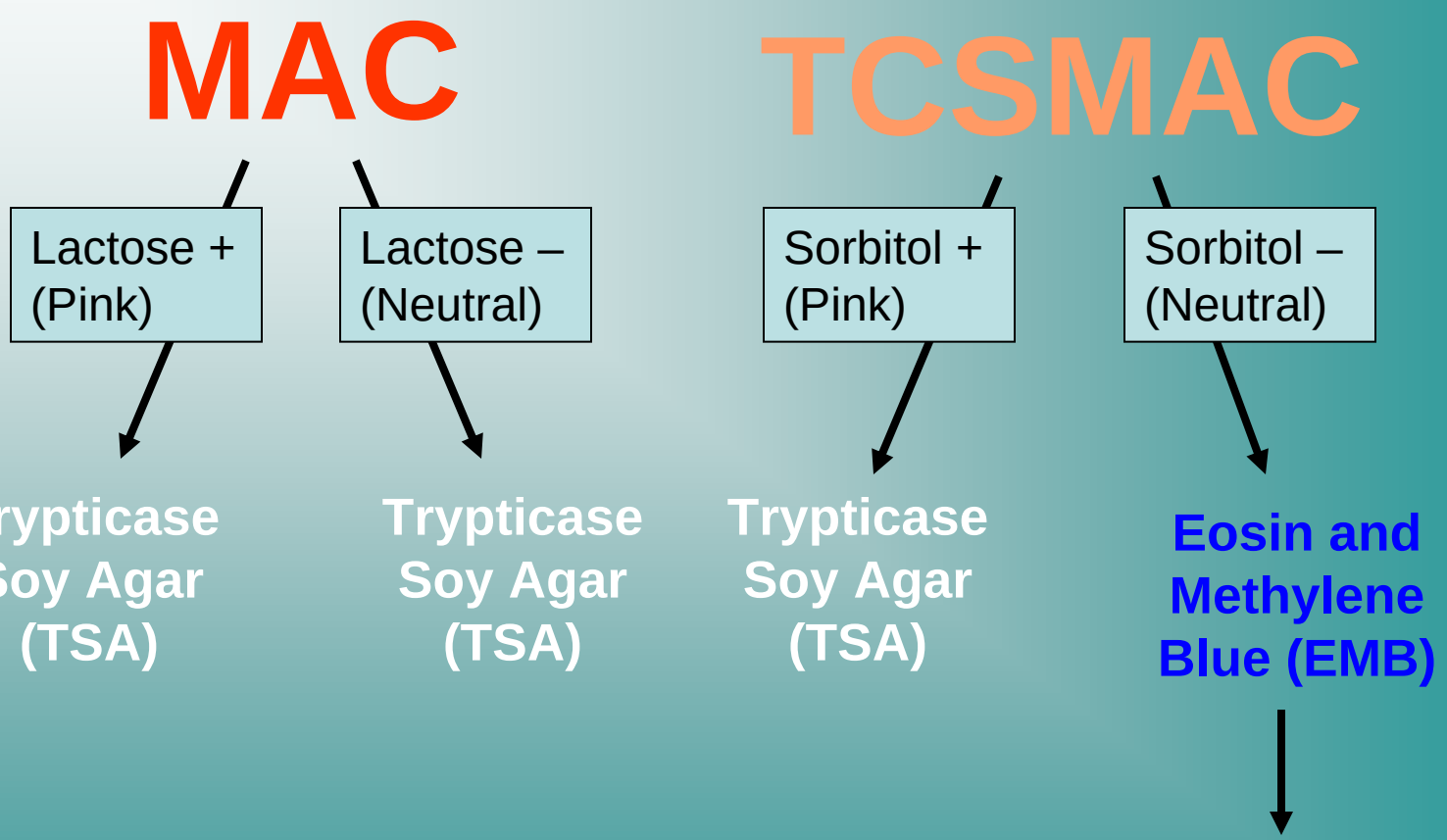
Samples were filtered using a membrane filtration system.

Primary Plating Media

MacConkey (MAC)

Tellurite-Cefixime Sorbitol MacConkey (TCSMAC)

Secondary Plating Media

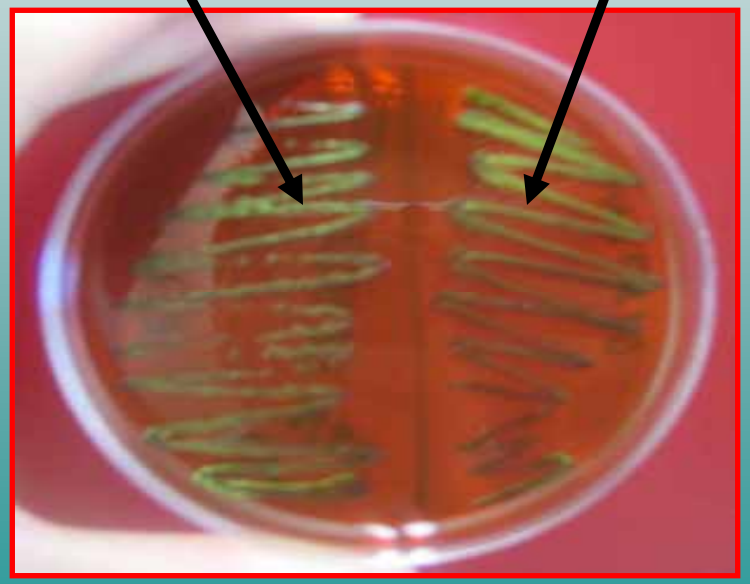


EMB Agar Plate

PSMUG Agar Plate

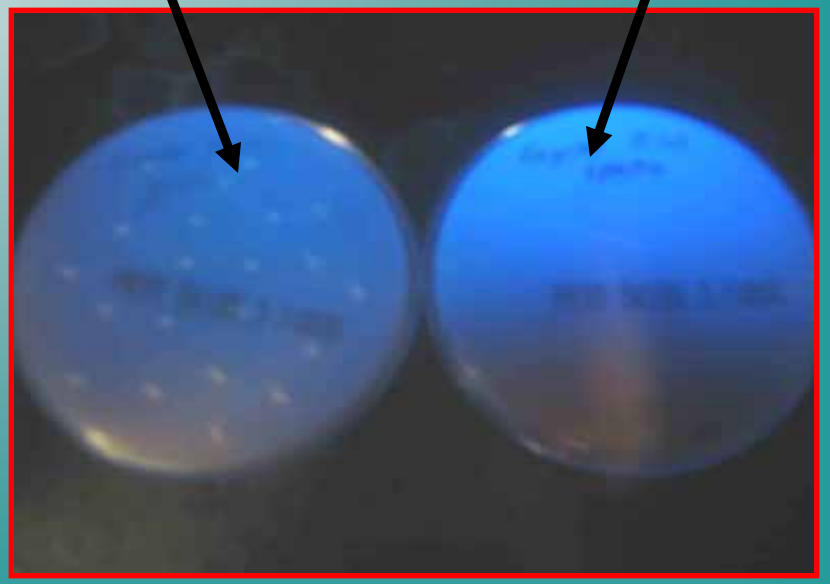
E. coli 0157: H7

E. coli



E. coli

E. coli 0157: H7



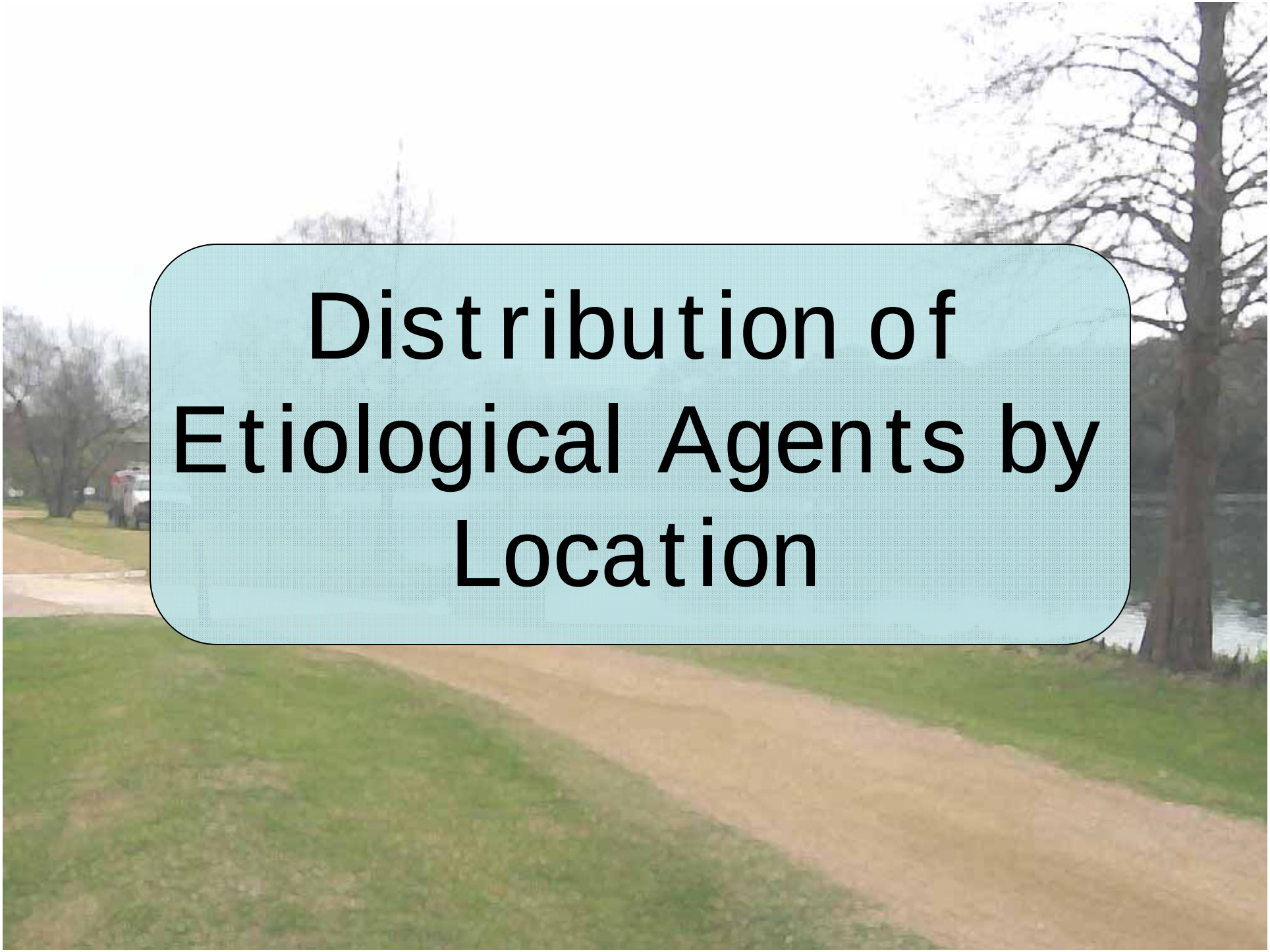
Biochemical Tests

API-20E Biochemical Test System

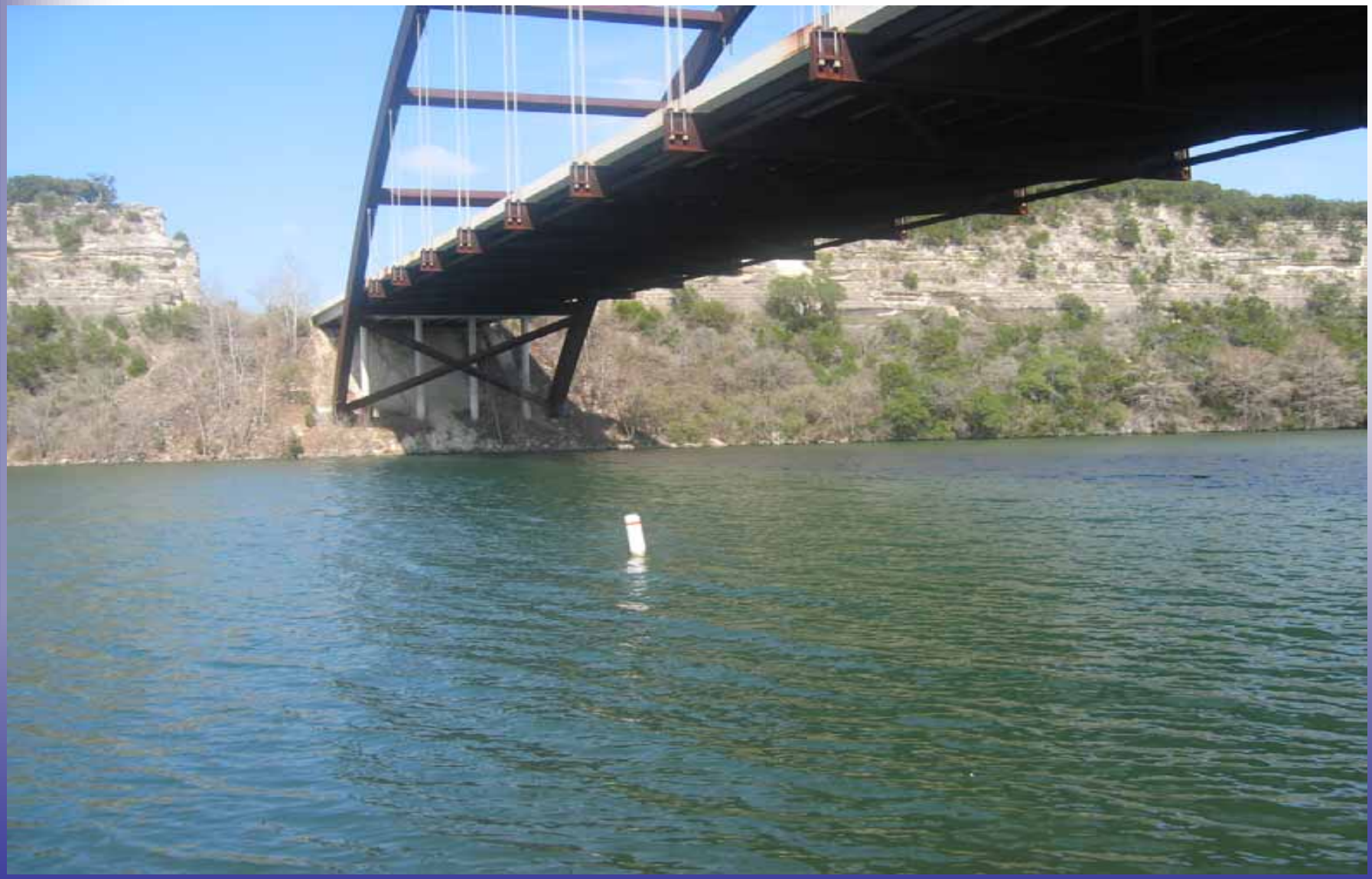
http://www.jlindquist.net/generalmicro/GBimages/API2.jpg

Confirmatory RIM Test

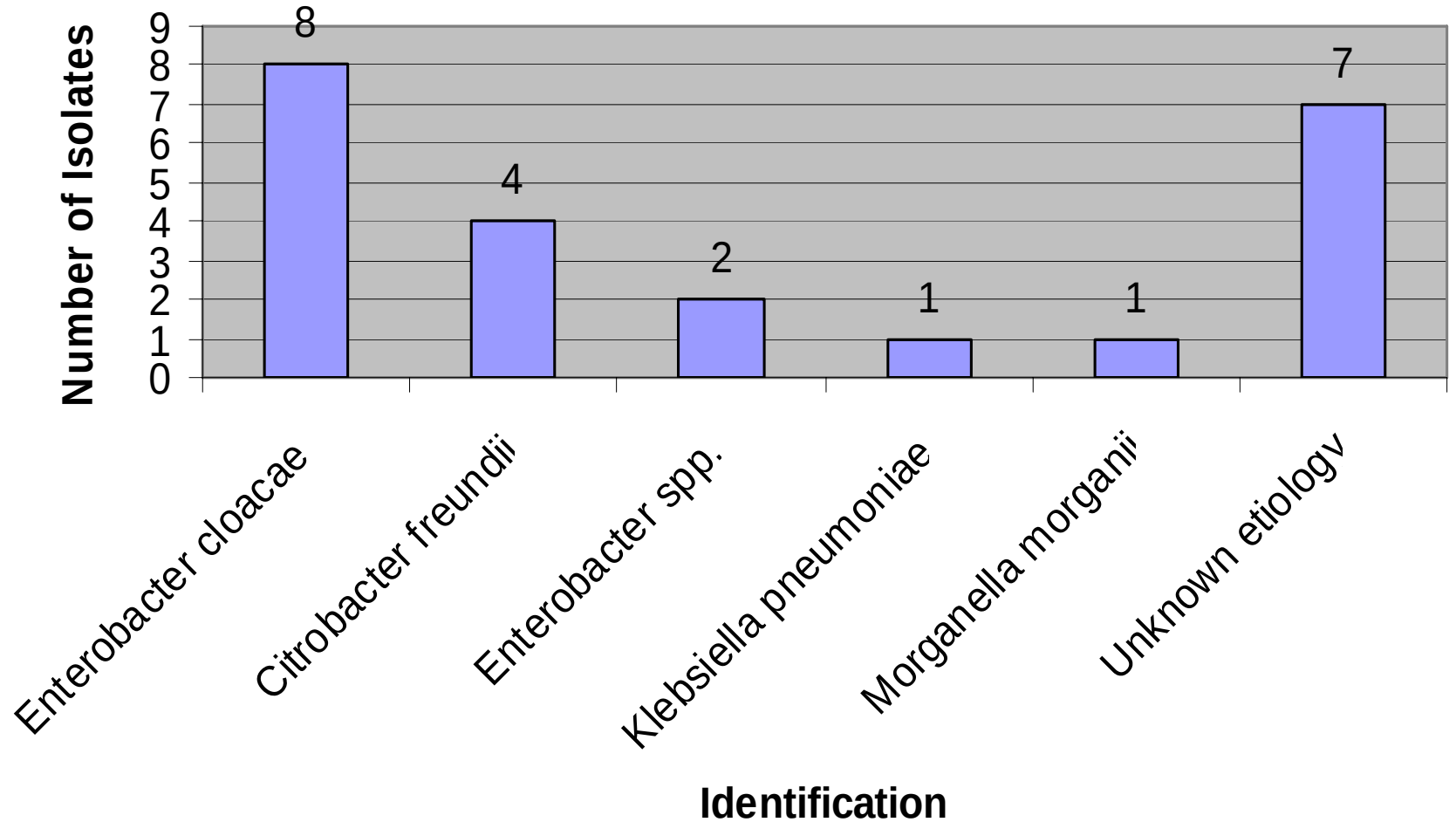

Results


Distribution of Etiological Agents by Location

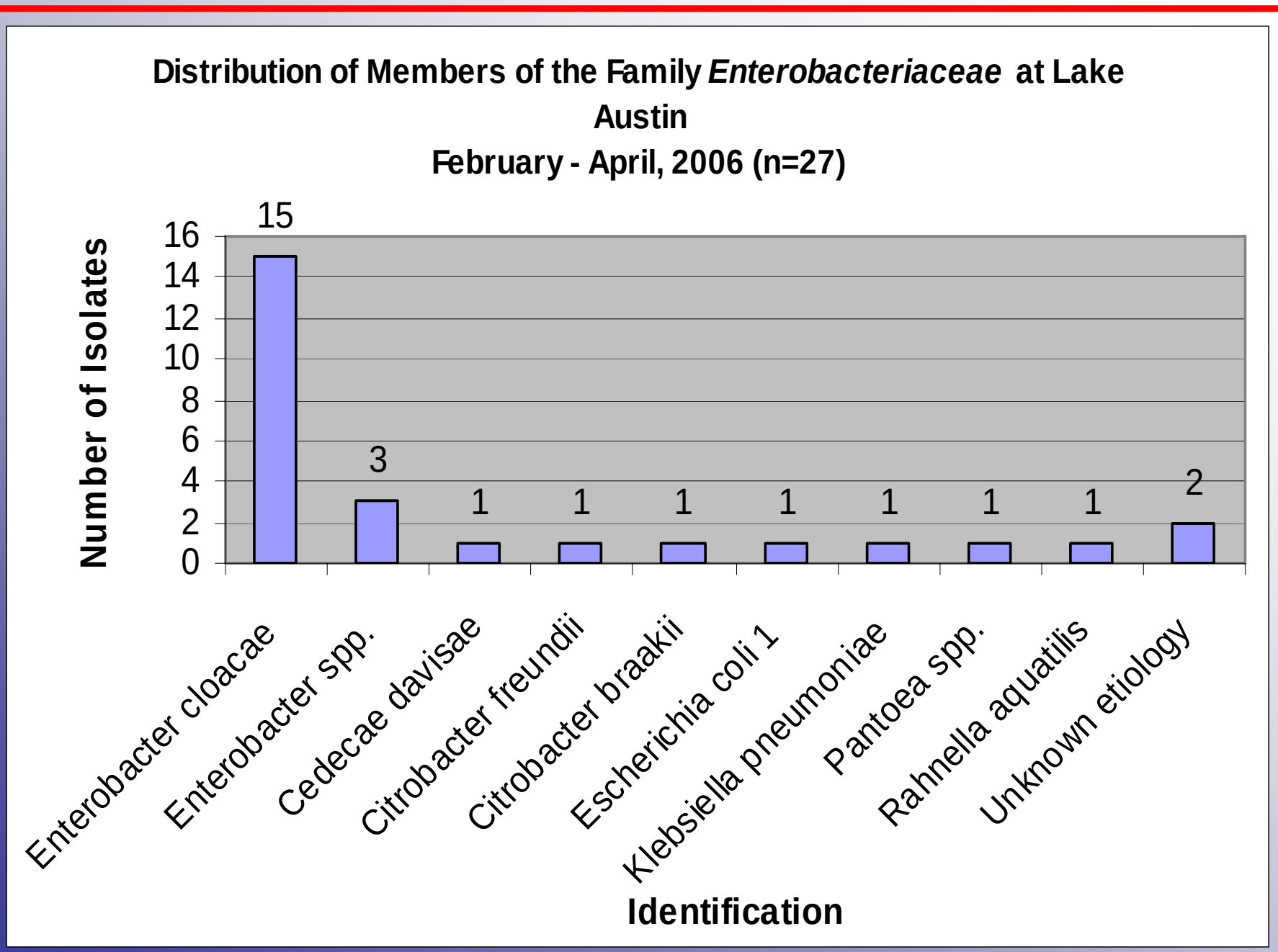
Pennybacker (360) Bridge



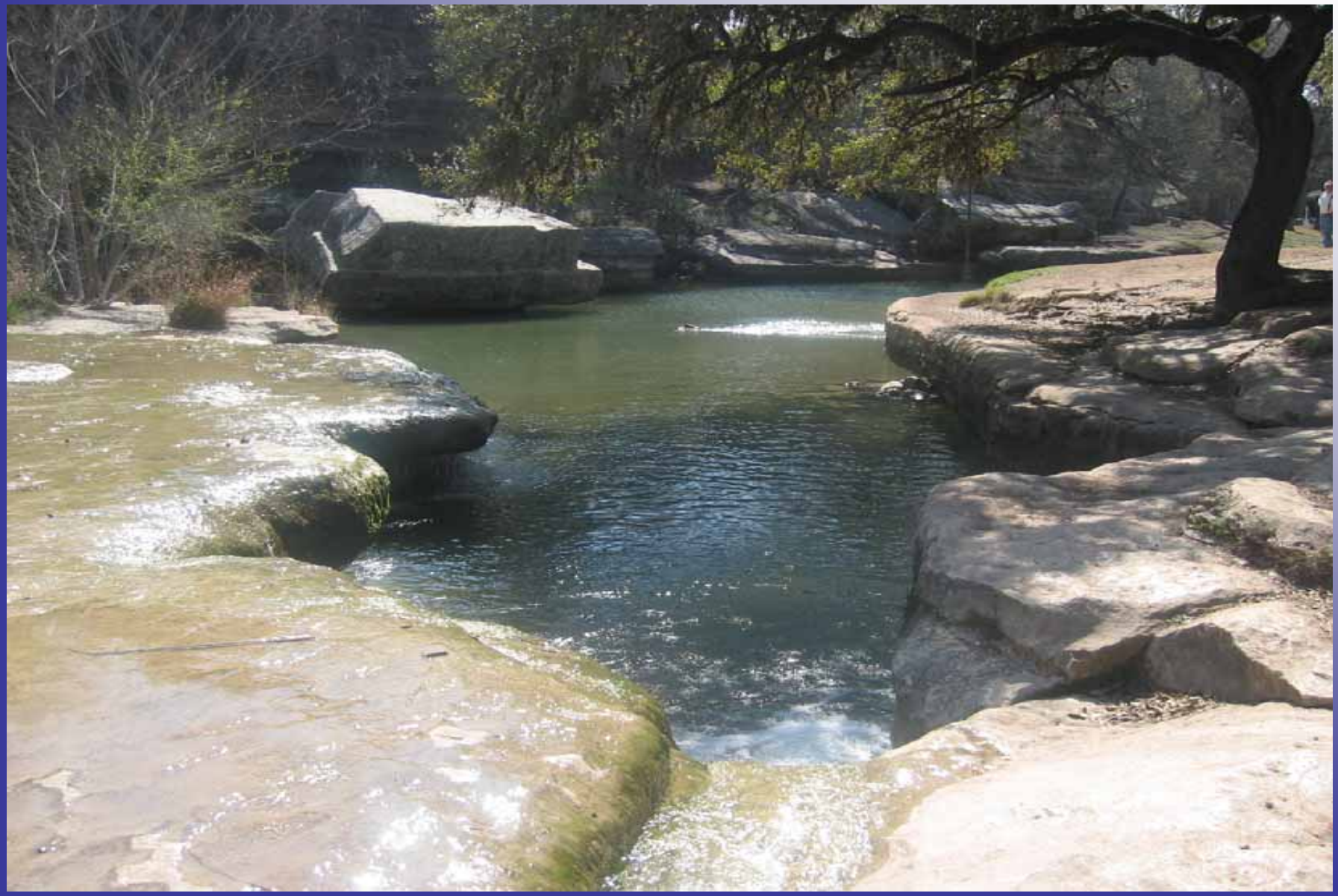
Distribution of Members of the Family *Enterobacteriaceae* at Pennybacker (360) Bridge
February - April, 2006 (n=23)

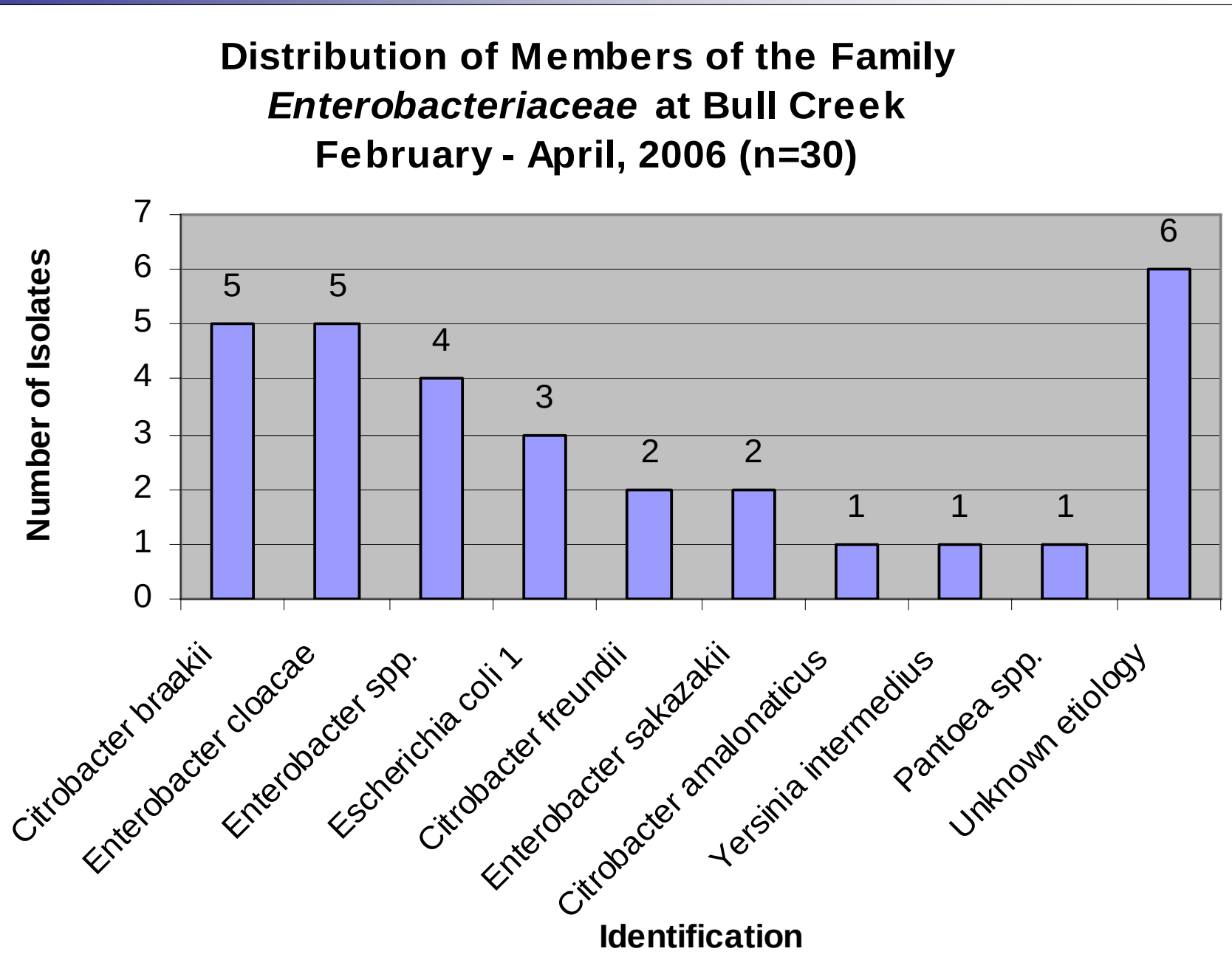


Lake Austin


Distribution of Members of the Family *Enterobacteriaceae* at Lake Austin
February - April, 2006 (n=27)
Number of Isolates
0
2
4
6
8
10
12
14
16
15
3
1
1
1
1
1
1
1
2
Enterobacter cloacae
Enterobacter spp.
Cedecae davisae
Citrobacter freundii
Citrobacter braakii
Escherichia coli 1
Klebsiella pneumoniae
Pantoea spp.
Rahnella aquatilis
Unknown etiology
Identification

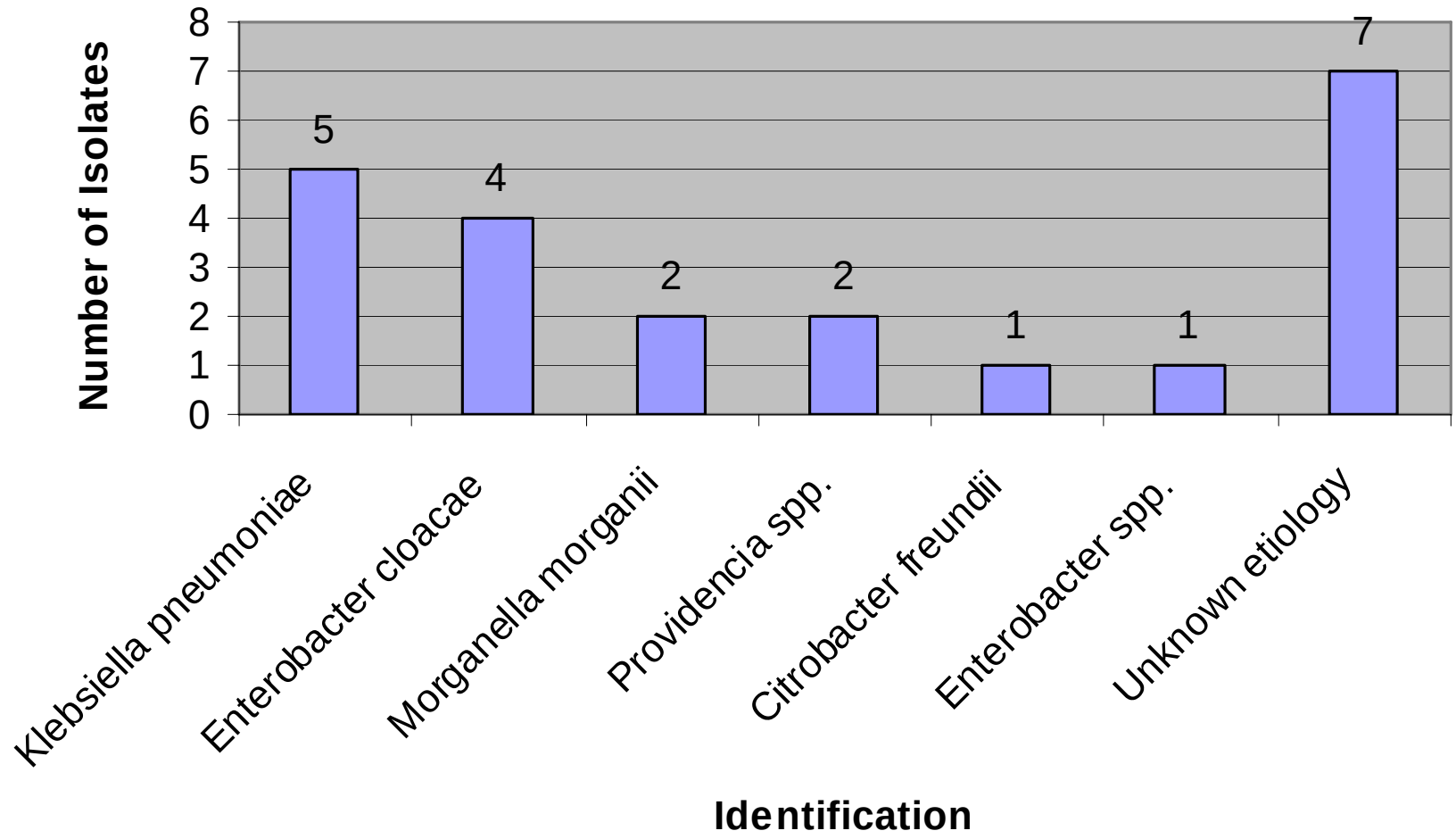
Bull Creek




Distribution of Members of the Family
Enterobacteriaceae at Bull Creek
February - April, 2006 (n=30)
Number of Isolates
0
1
2
3
4
5
6
7
5
5
4
3
2
2
1
1
1
6
Citrobacter braakii
Enterobacter cloacae
Enterobacter spp.
Escherichia coli 1
Citrobacter freundii
Enterobacter sakazakii
Citrobacter amalonaticus
Yersinia intermedius
Pantoea spp.
Unknown etiology
Identification

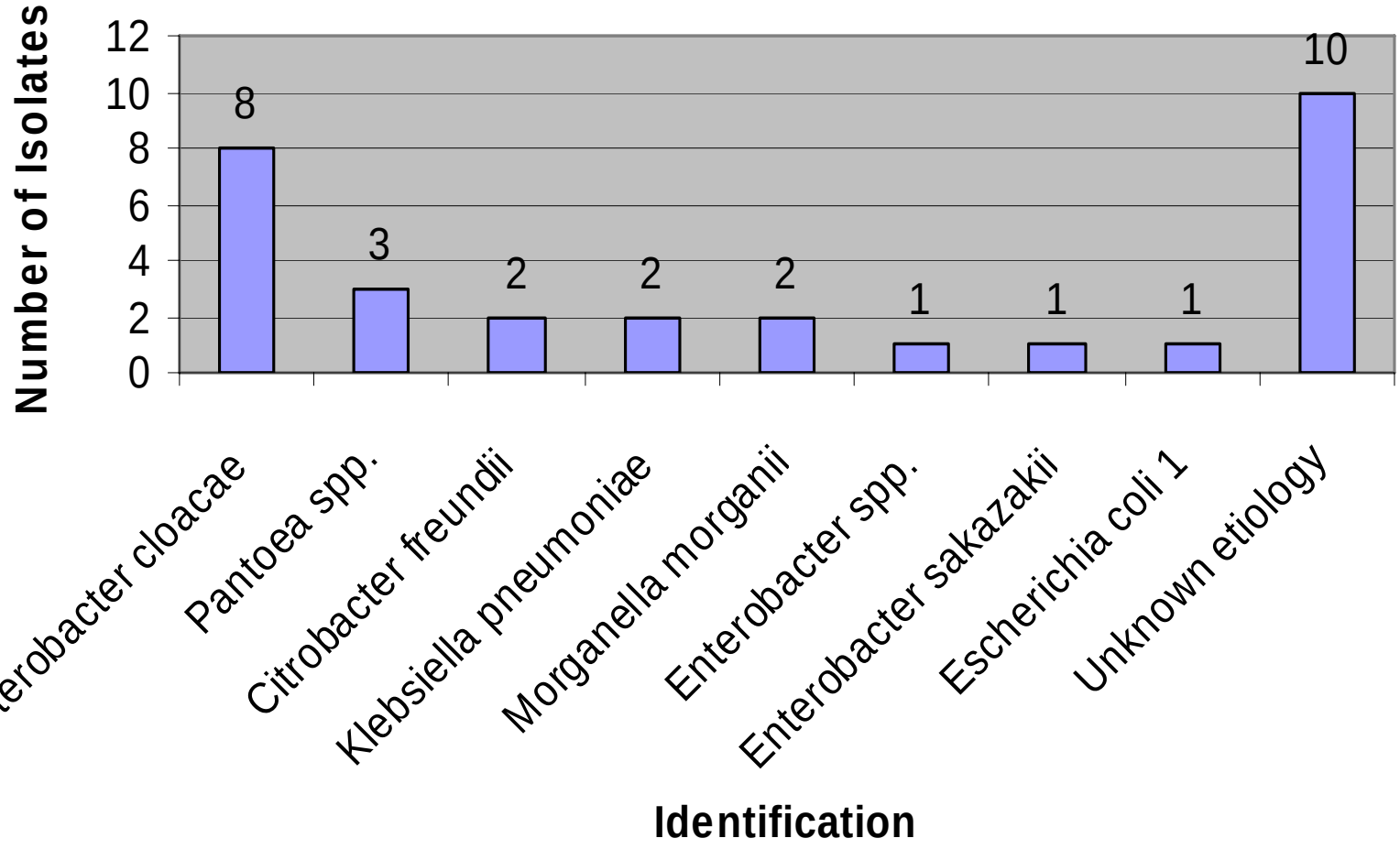
Barton Spring Pool

Distribution of Members of the Family *Enterobacteriaceae* at Barton Spring Pool February - April, 2006 (n=22)



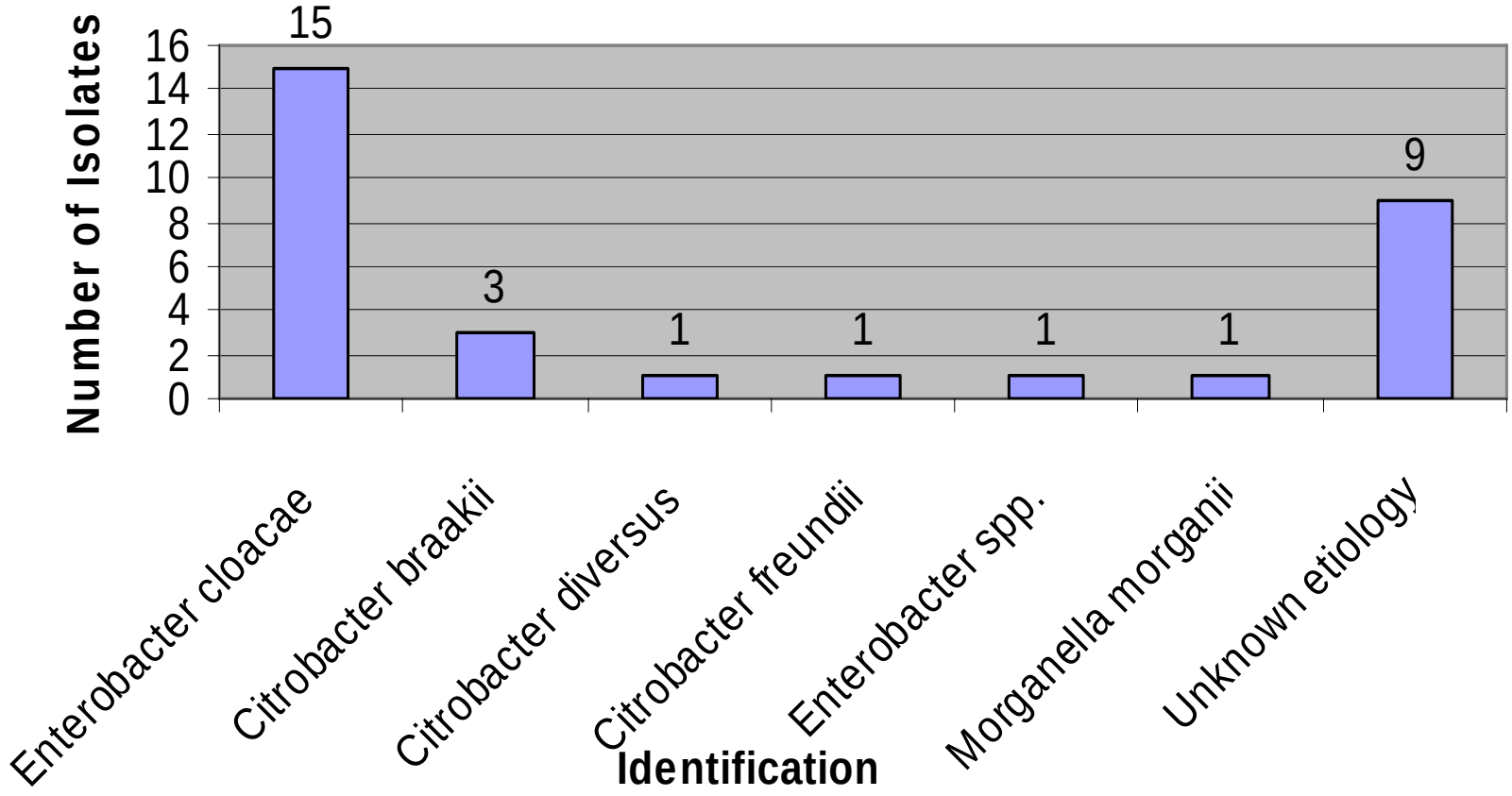
South of Barton Spring Pool

Distribution of Members of the Family *Enterobacteriaceae* at South Barton Spring
February - April, 2006 (n=30)

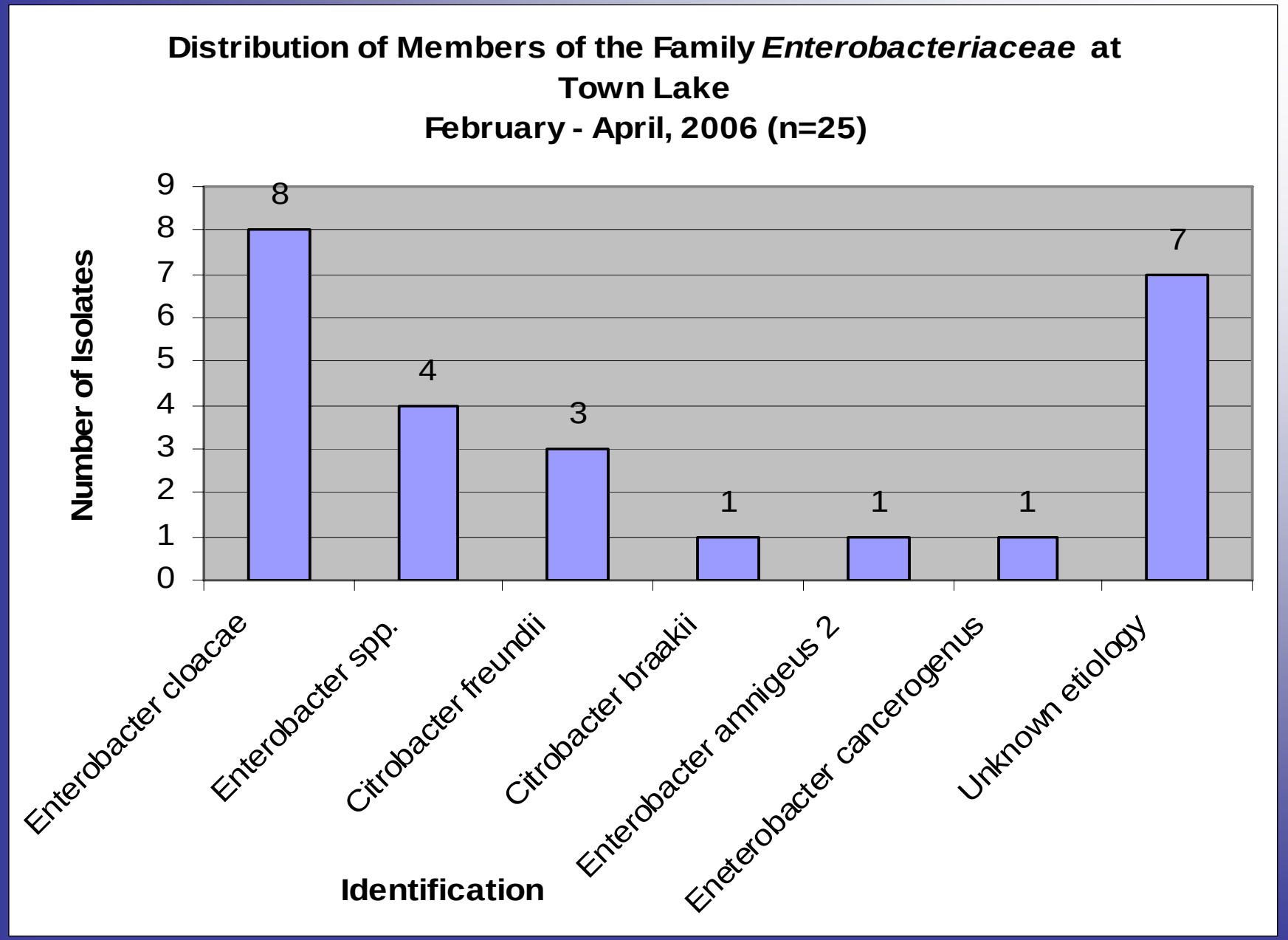


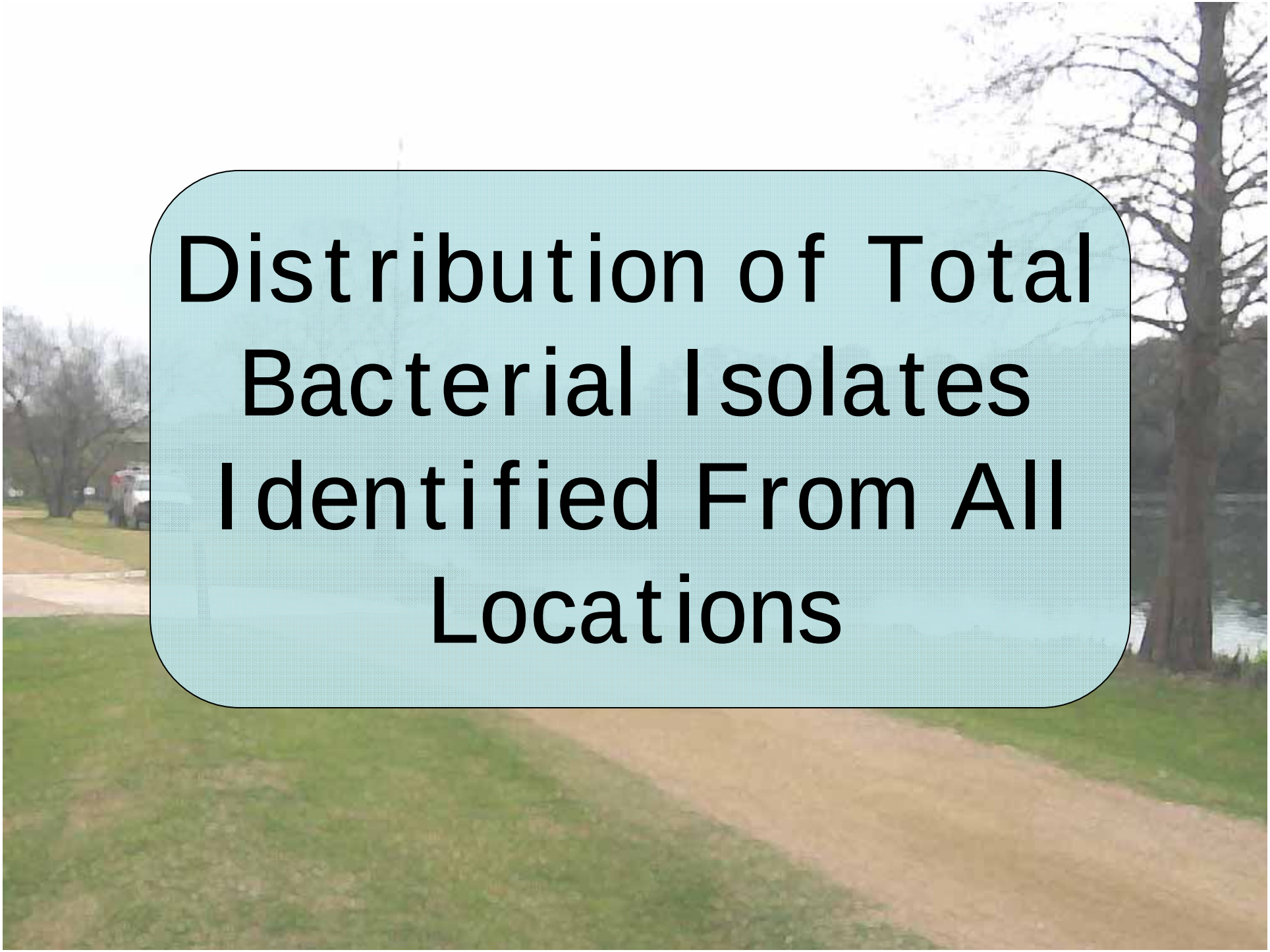
Campbell's Hole

Distribution of Members of the Family *Enterobacteriaceae* at Campbell's Hole
February - April, 2006 (n=31)

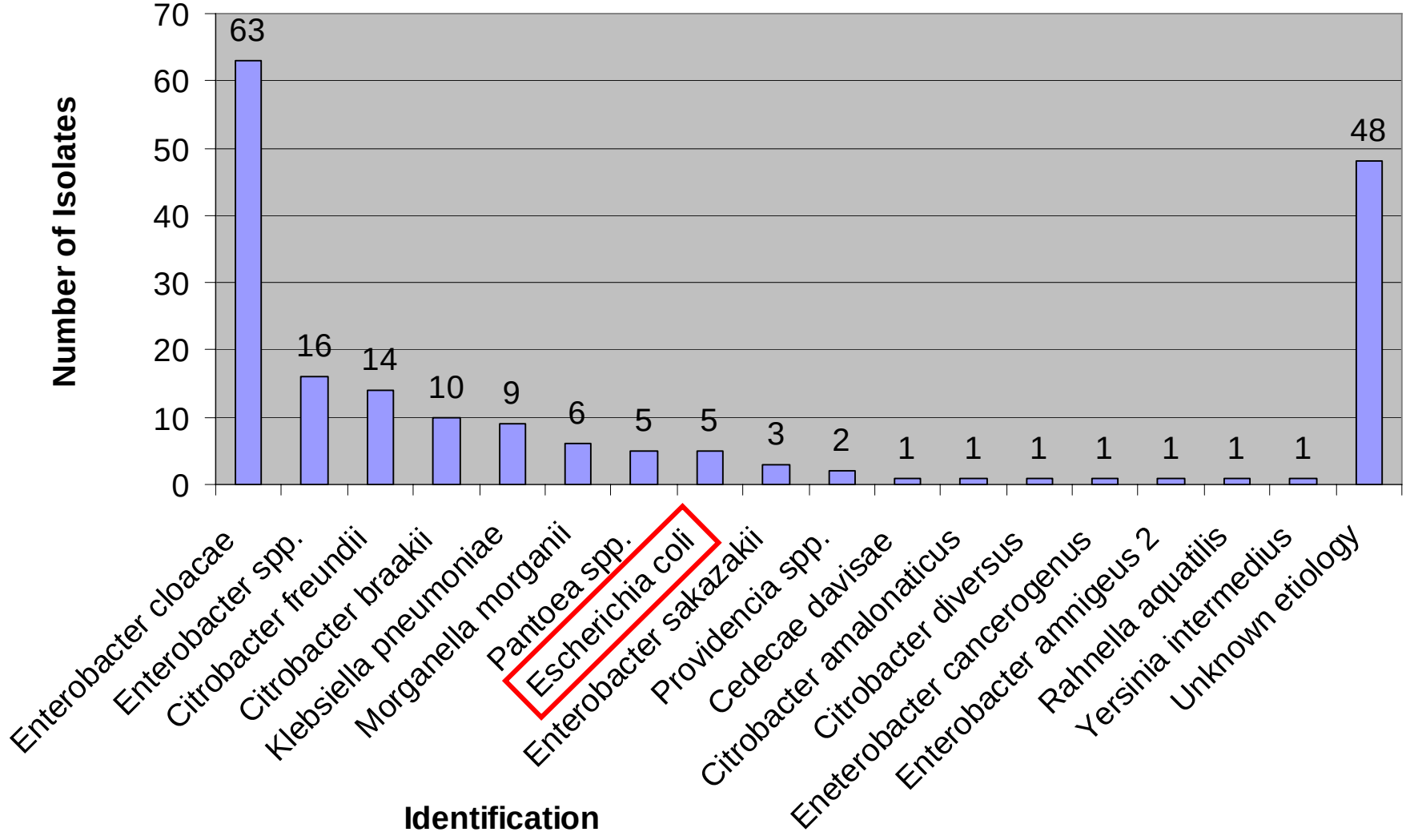


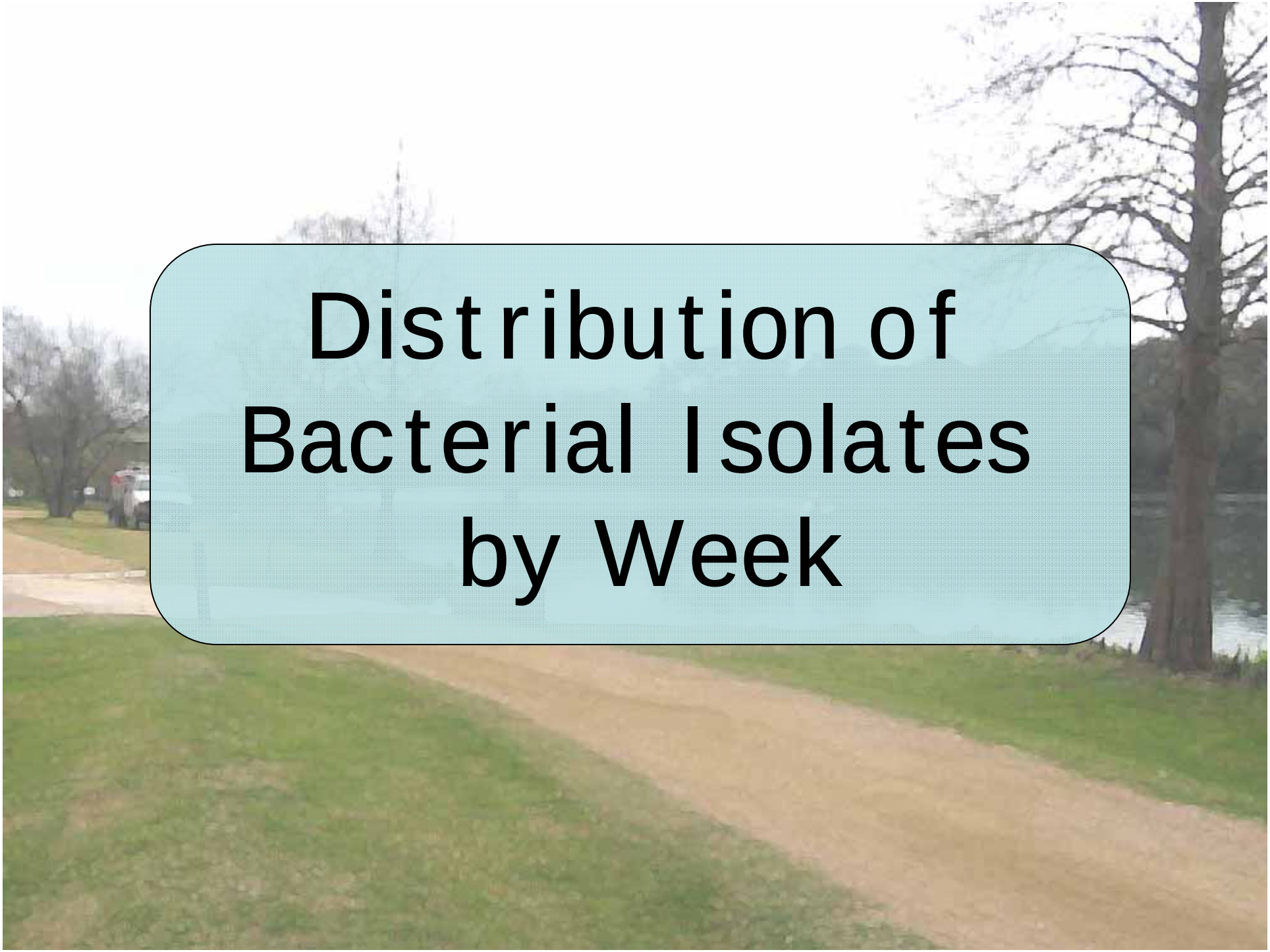
Town Lake


Distribution of Members of the Family *Enterobacteriaceae* at Town Lake
February - April, 2006 (n=25)
Number of Isolates
0
1
2
3
4
5
6
7
8
9
8
4
3
1
1
1
7
Enterobacter cloacae
Enterobacter spp.
Citrobacter freundii
Citrobacter braakii
Enterobacter amnigeus 2
Eneterobacter cancerogenus
Unknown etiology
Identification


Distribution of Total Bacterial Isolates Identified From All Locations

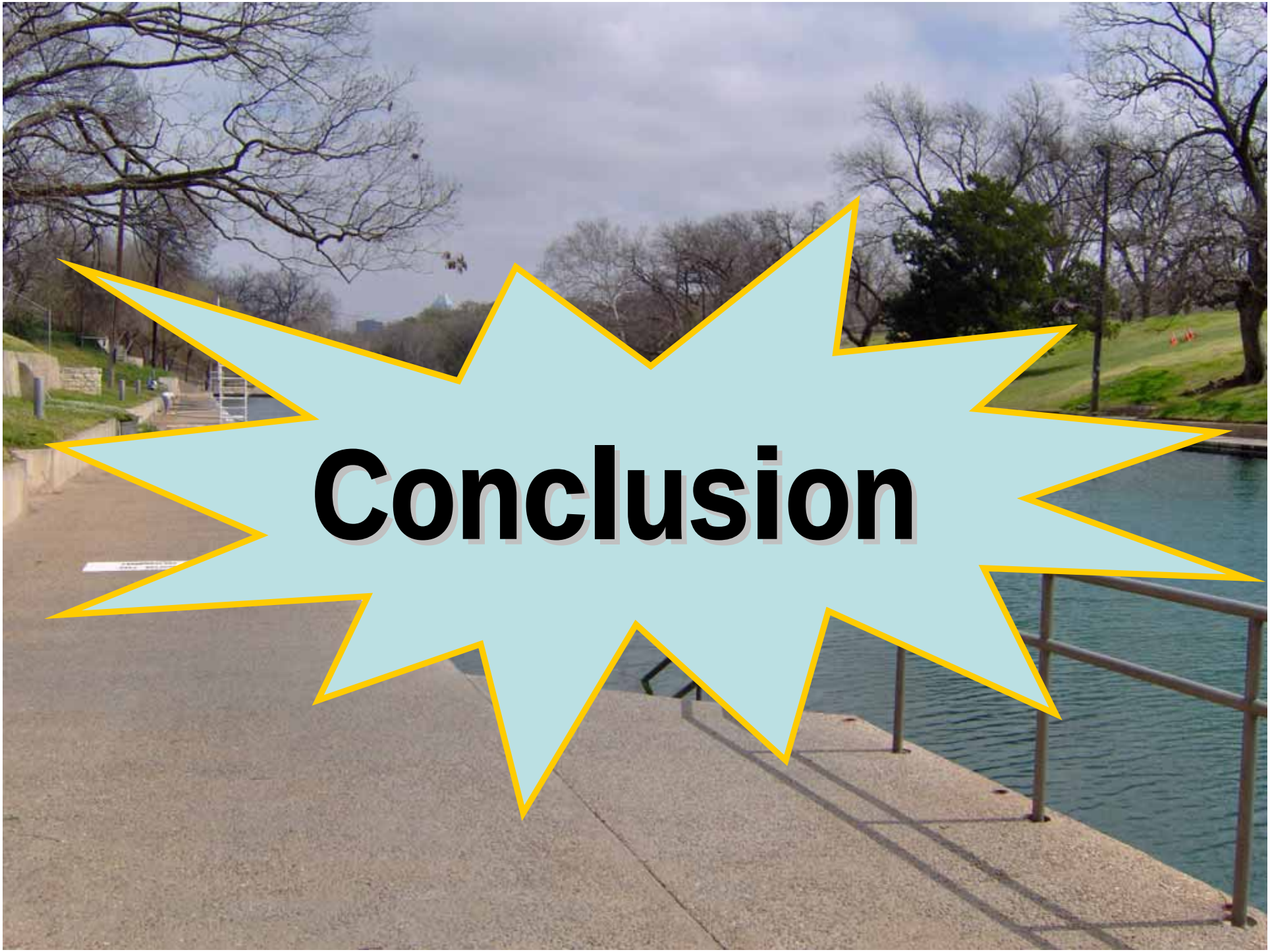
Distribution of Members of the *Enterobacteriacae* Family Isolated from Recreational Waters in and Around Austin, TX February - April, 2006 (n=188)




Distribution of
Bacterial Isolates
by Week

Distribution of Total Etiological Agents Found Two or More Times over a 7 Weeks Period

	E. cloacae	*C. freundii*	*C. diversus*	*C. braakii*	*K. pneumoniae*	*M. morganii*	*P. spp.*
Town Lake	5	2	2				
Campbell's Hole	7		2				
Barton Springs	3						
South of Barton Springs	5	3					
Bull Creek	5	2		2	2	2	2
Pennybacker Bridge	3	2					
Lake Austin	7	2		2	2		
Total	35	11	4	4	4	2	2


Conclusion

Conclusion

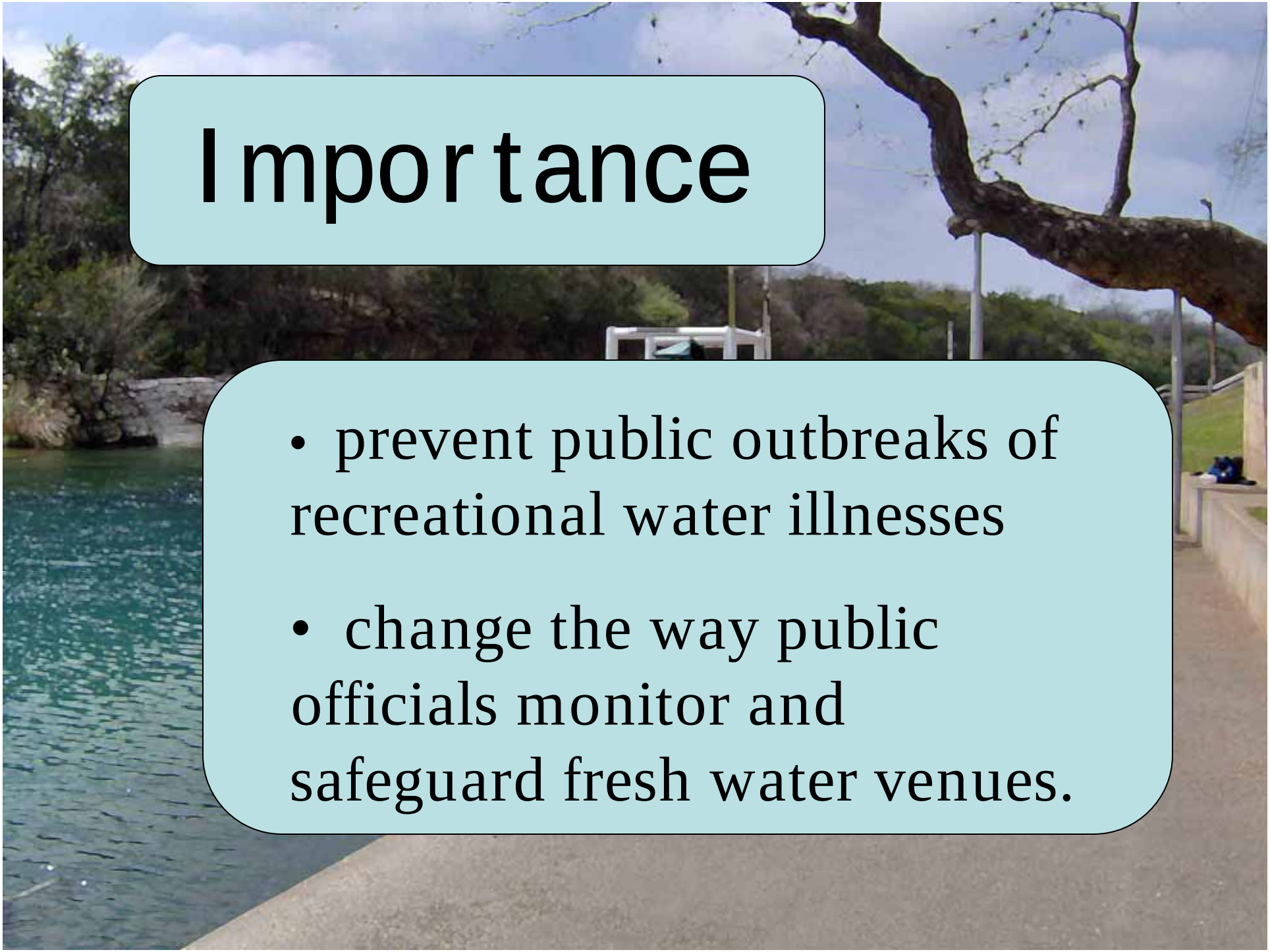
- **E. coli (5)**
  - Bull Creek (3)
  - Lake Austin (1)
  - South of Barton Spring (1)
  - None were O157:H7
- **No other frank pathogens recovered**

Conclusion (cont.)

- *E. cloacae* **– 63 (34%)**
  - Most frequent isolate
  - Consistently isolated over the 7 weeks period
- *C. freundii* **- 14 (7%)**
  - Second most frequent isolate
  - Less consistently isolated than *E. cloacae* from each site week to week
- **Unknown etiology - 48 (26%)**

Limitations

- Incubation at 37 C did not allow differentiation between coliforms and “fecal” coliforms.
- Difficult to determine significance of isolates (other than *E. coli*)
  - *C. freundii* and *E. cloacae*
  - Opportunistic human pathogens or may also be harmless environmental microorganisms.



Importance

- prevent public outbreaks of recreational water illnesses
- change the way public officials monitor and safeguard fresh water venues.


Future
Studies

Future Studies

- Determine if coliforms present are of human origin by incubating isolates at 44ºC.
- Repeat the study to quantify number of coliforms present.
- Longer surveys of recreational water locations.
- More locations around Austin, TX.
- Correlation between the number of etiological agents found from one location at different seasons of the year.


Acknowledgments

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Thank You

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