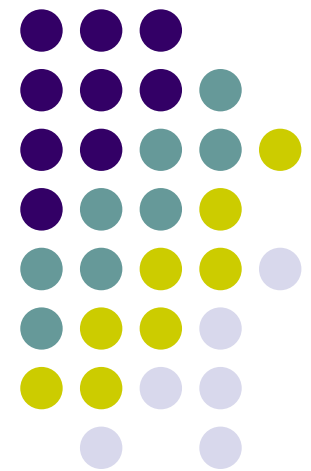


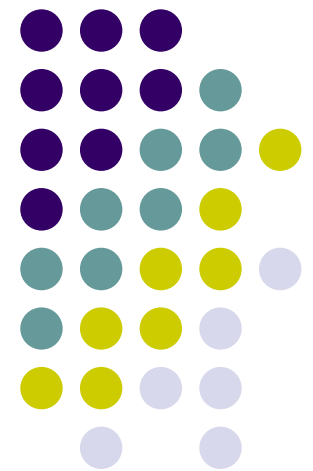
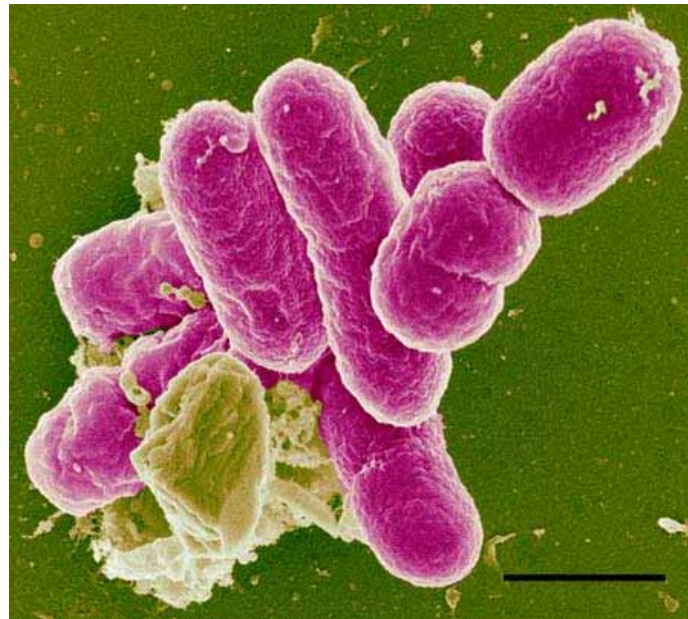
Analysis of *Shigella* strains by Pulsed Field Gel Electrophoresis

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Introduction



Shigella species

- Family: *Enterobacteriaceae*
- Causes bacillary dysentery
- 4 Serogroups:
 - *S. dysenteriae* (serogroup A)
 - *S. flexneri* (serogroup B)
 - *S. boydii* (serogroup C)
 - *S. sonnei* (serogroup D)



Shigella spp.

□ *S. dysenteriae*

- Causes the most severe illness
- Produces cytotoxin, Shiga toxin
- Main cause of outbreaks in developing countries

□ *S. flexneri*

- Found most commonly in developing countries

□ *S. sonnei*

- Predominant in developed countries
- Increase in prevalence over last few years

□ *S. boydii*

- Rarely seen outside the Indian subcontinent

Characteristics

- Gram negative, facultative, anaerobic rods
- Low infectious dose—10-100 bacteria
- Transmission
 - Human-to-human
 - Contaminated food
 - Drinking water
 - Swimming pools
 - Flies



Bacillary dysentery

- ❑ Triad of symptoms: cramps, urgency and painful defecation, and frequent, small volume, bloody, mucoid diarrhea
- ❑ Incubation period: 12 hours to 2 days
- ❑ Other symptoms include fever, malaise, anorexia, nausea, vomiting, and myalgia

Clinical overview

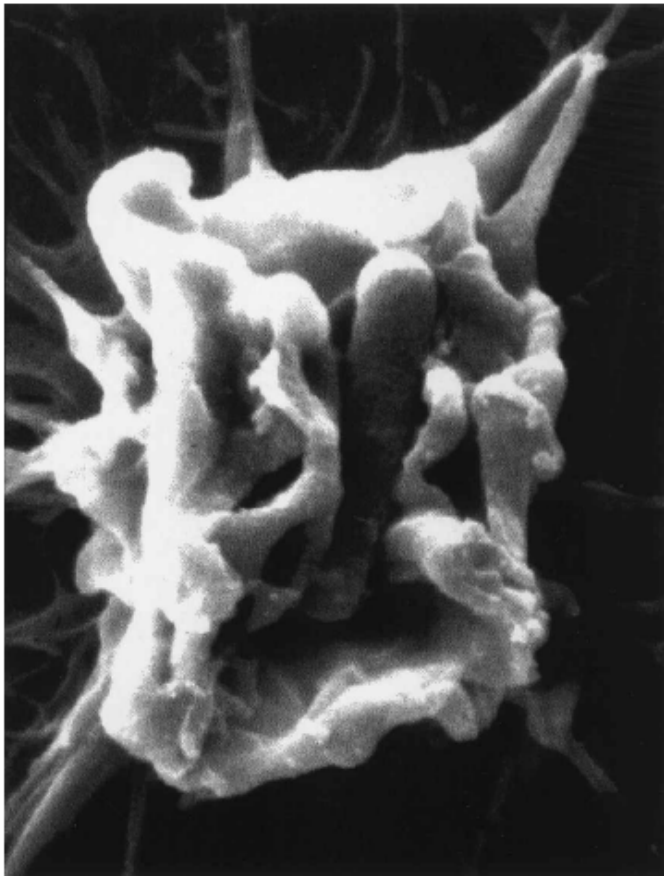
- Healthy adults—self-limiting disease
- Children and elderly—severe dehydration and sometimes death
- Extraintestinal complications:
 - Bacteremia
 - Septicemia
 - Neurological manifestations

Epidemiology

- Highest incidence in young children 1-5 years of age
- Child daycare centers
- Sexually transmitted disease among male homosexuals



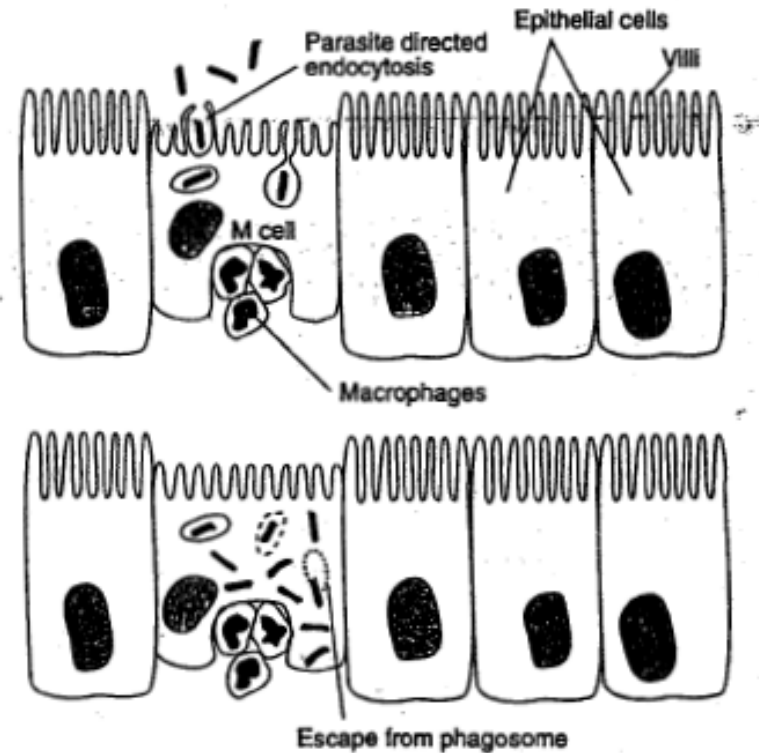
Virulence Factors



- ❑ Invasion plasmid antigens (Ipa proteins)
- ❑ Mxi-Spa proteins
- ❑ IcsA and IcsB proteins
- ❑ Lipopolysaccharide (LPS endotoxin)
- ❑ Shiga toxin (only produced by *S. dysenteriae*)

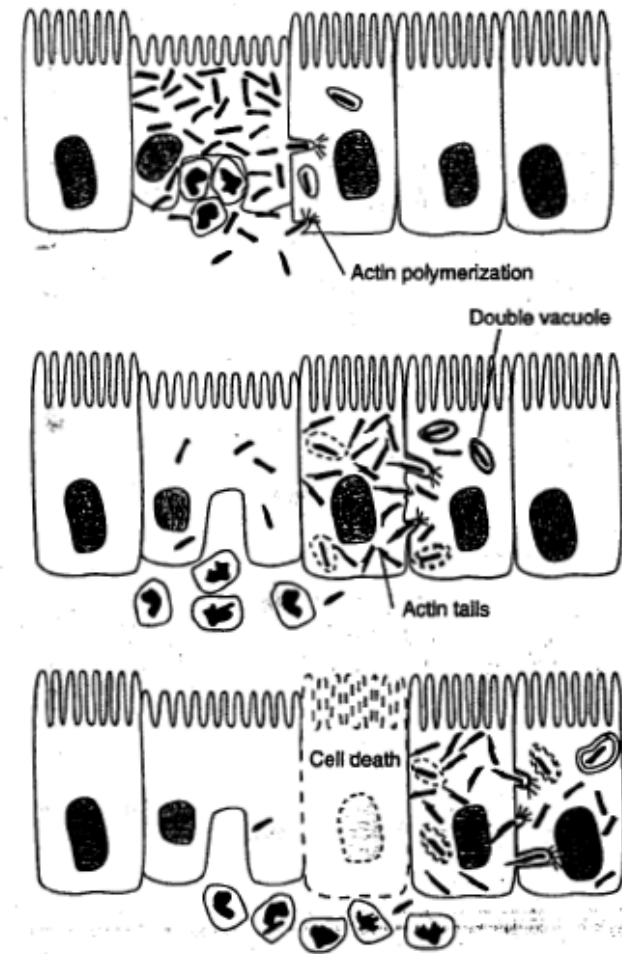
Pathogenesis

1. Enter host
2. Attach and invade
3. Enter host cell
4. Escape phagosome



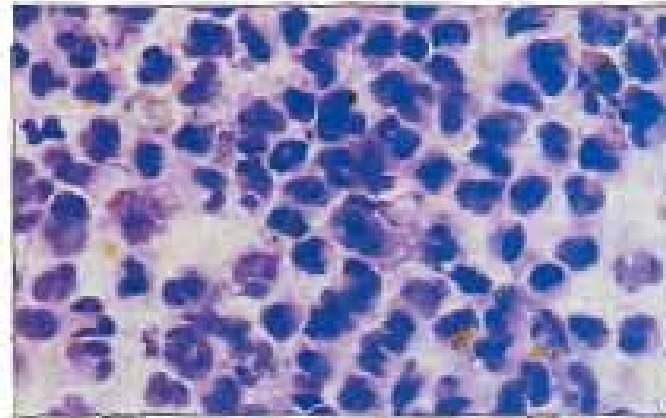
Pathogenesis

5. Multiply in cytoplasm
6. Spread
7. Trigger inflammatory response
8. Damage to host tissue



Diagnosis

- ❑ Culture and isolation of organism from stool specimens
- ❑ Leukocytes in stool
- ❑ Biochemical and serological tests
- ❑ Pulsed field gel electrophoresis (PFGE) to fingerprint bacterial DNA

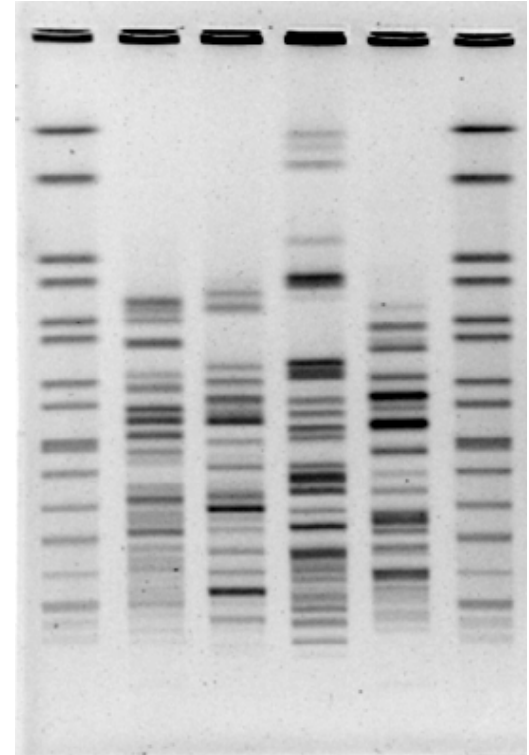


Pulsed Field Gel Electrophoresis (PFGE)

- ❑ Molecular genetic method for subtyping bacteria
- ❑ Compare isolates of same species
- ❑ Detect minor differences in genomes
- ❑ Most useful for analysis of large DNA genomes (such as bacteria)

Pulsed Field Gel Electrophoresis (PFGE)

- ❑ DNA is trapped in agarose plugs and digested with restriction endonucleases
- ❑ DNA fragments subject to electrophoresis on agarose gels
- ❑ Fragments separate according to size



Treatment

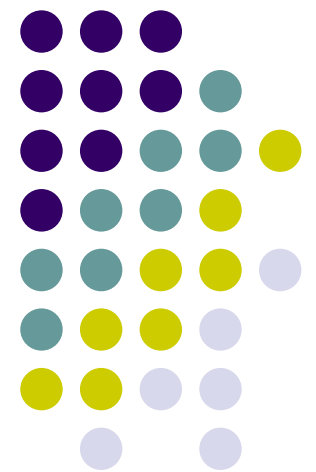
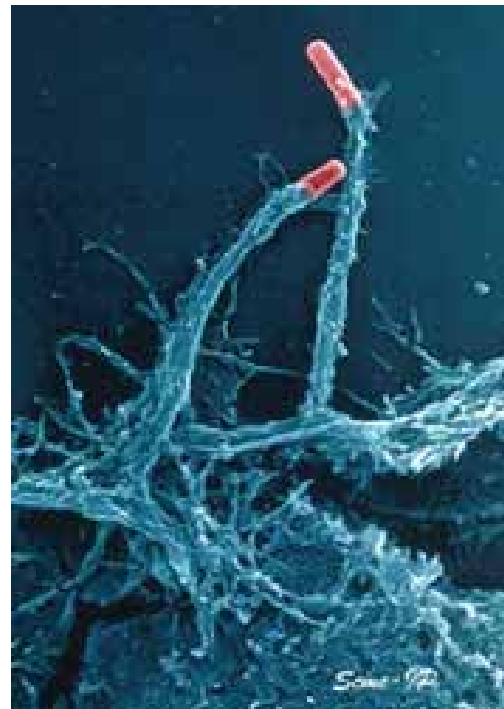
- ❑ Oral rehydration therapy
- ❑ Antibiotics
 - Increasing antibacterial resistance
- ❑ Do not use agents that inhibit gut motility



Purpose

- Analyze strains of *Shigella* by PFGE
 - Quality control specimens from ATCC
 - 2003 clinical isolates received by the laboratory at the Texas Department of State Health Services (TDSHS) in Austin

Methods

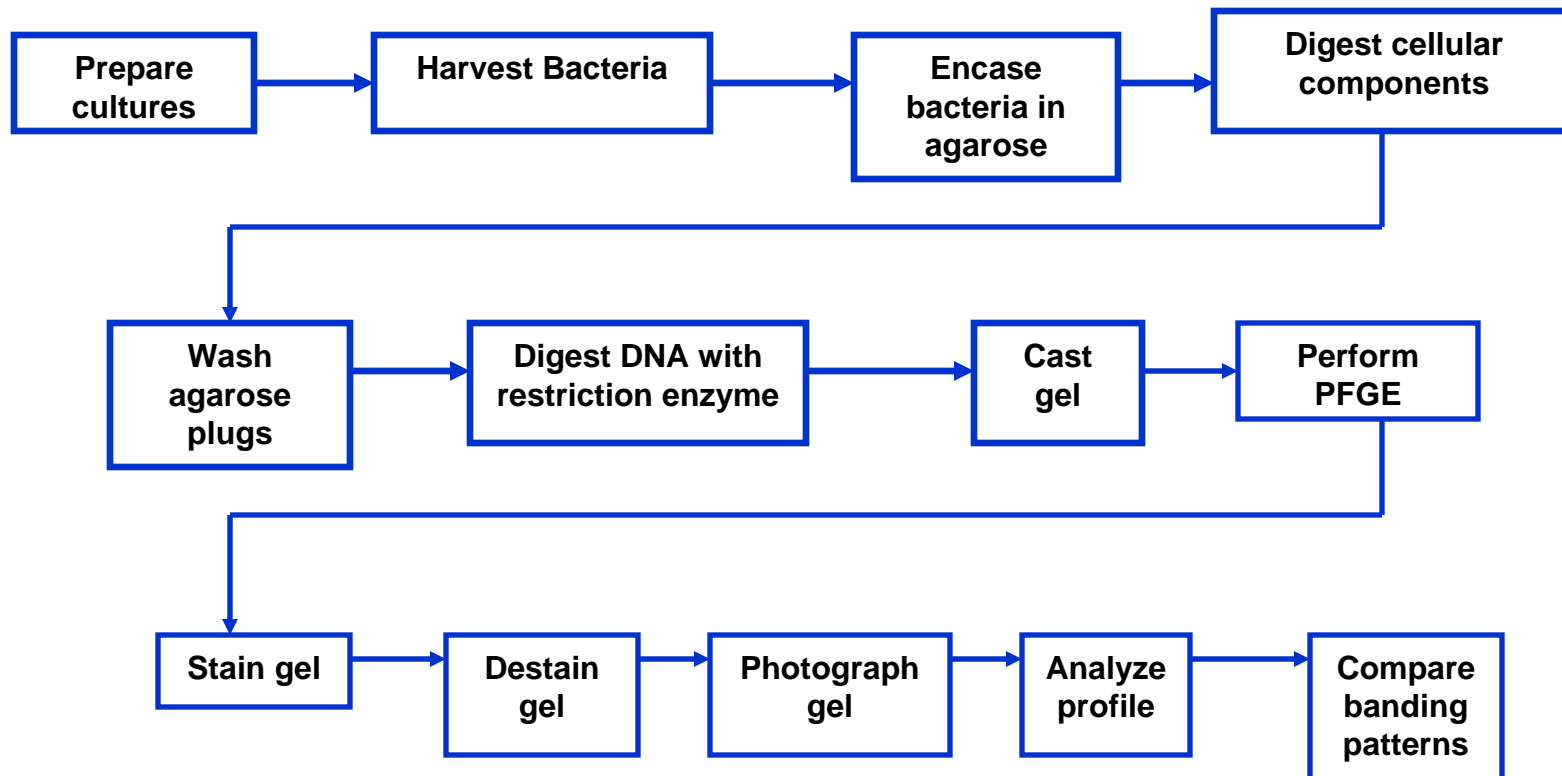


Sources

- American Type Culture Collection (ATCC)
 - Quality control specimens (42 total)
 - Included: *S. boydii* (17), *S. dysenteriae* (12), *S. flexneri* (11), and *S. sonnei* (2)

- Patient Isolates from 2003 (73)
 - Received at the Molecular Biology Laboratory at the Texas Department of State Health Services (TDSHS) from all over the state in 2003
 - Included: *S. flexneri* (66) and *S. boydii* (7)

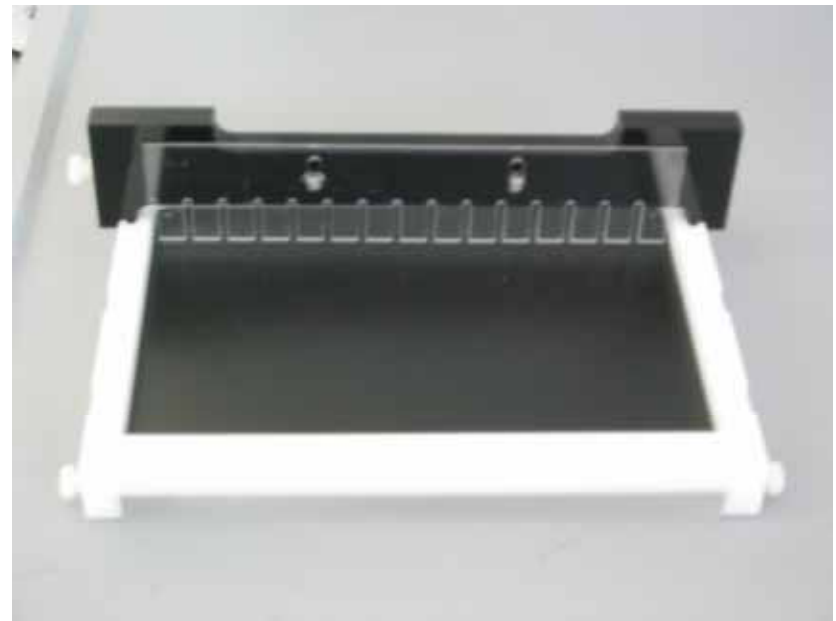
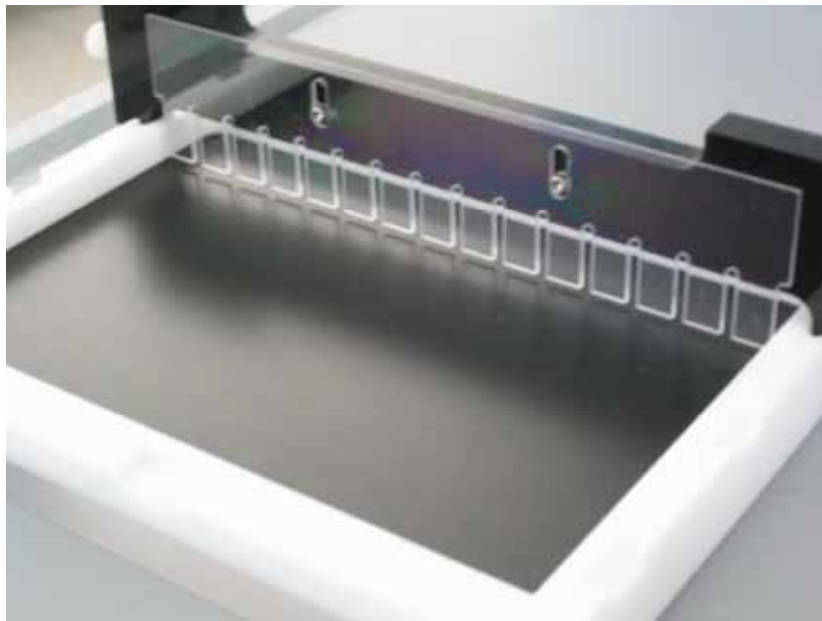
Methods Used to Analyze *Shigella* using PFGE



Washing agarose plugs



Agarose Gel Mold



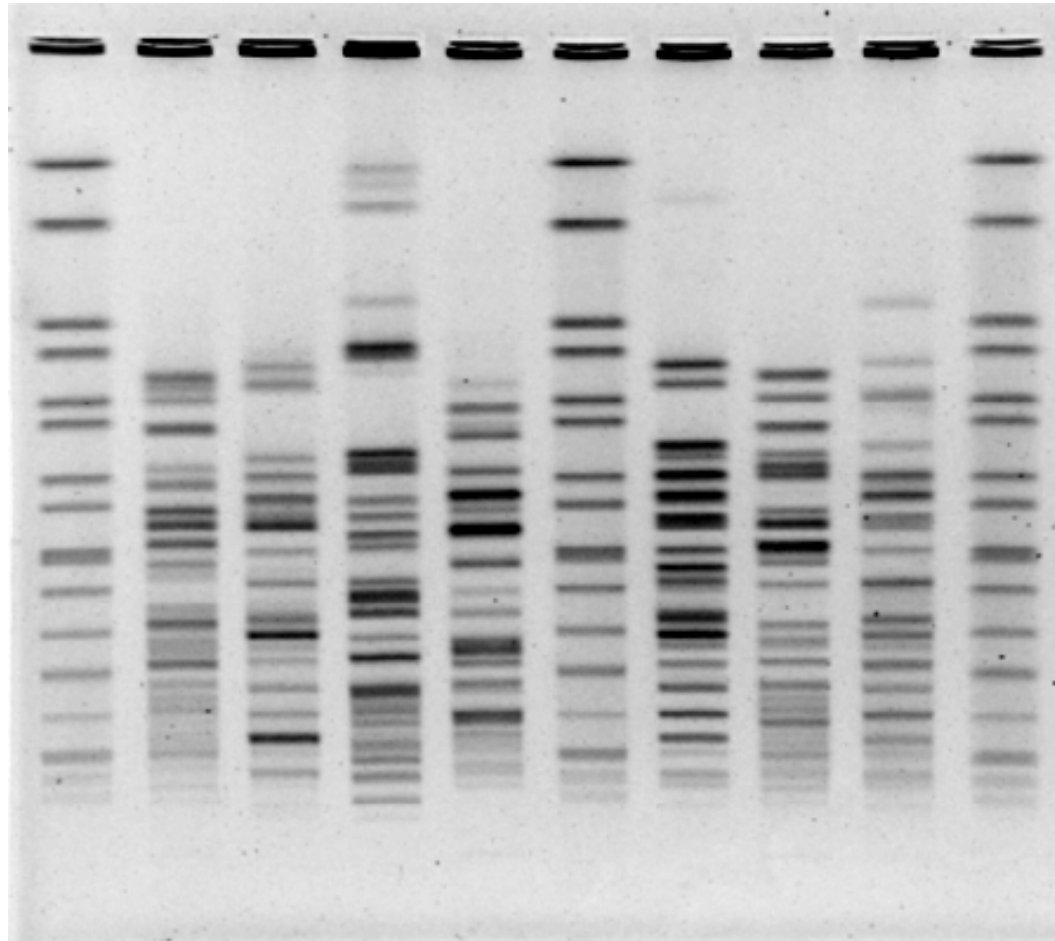
Electrophoresis system



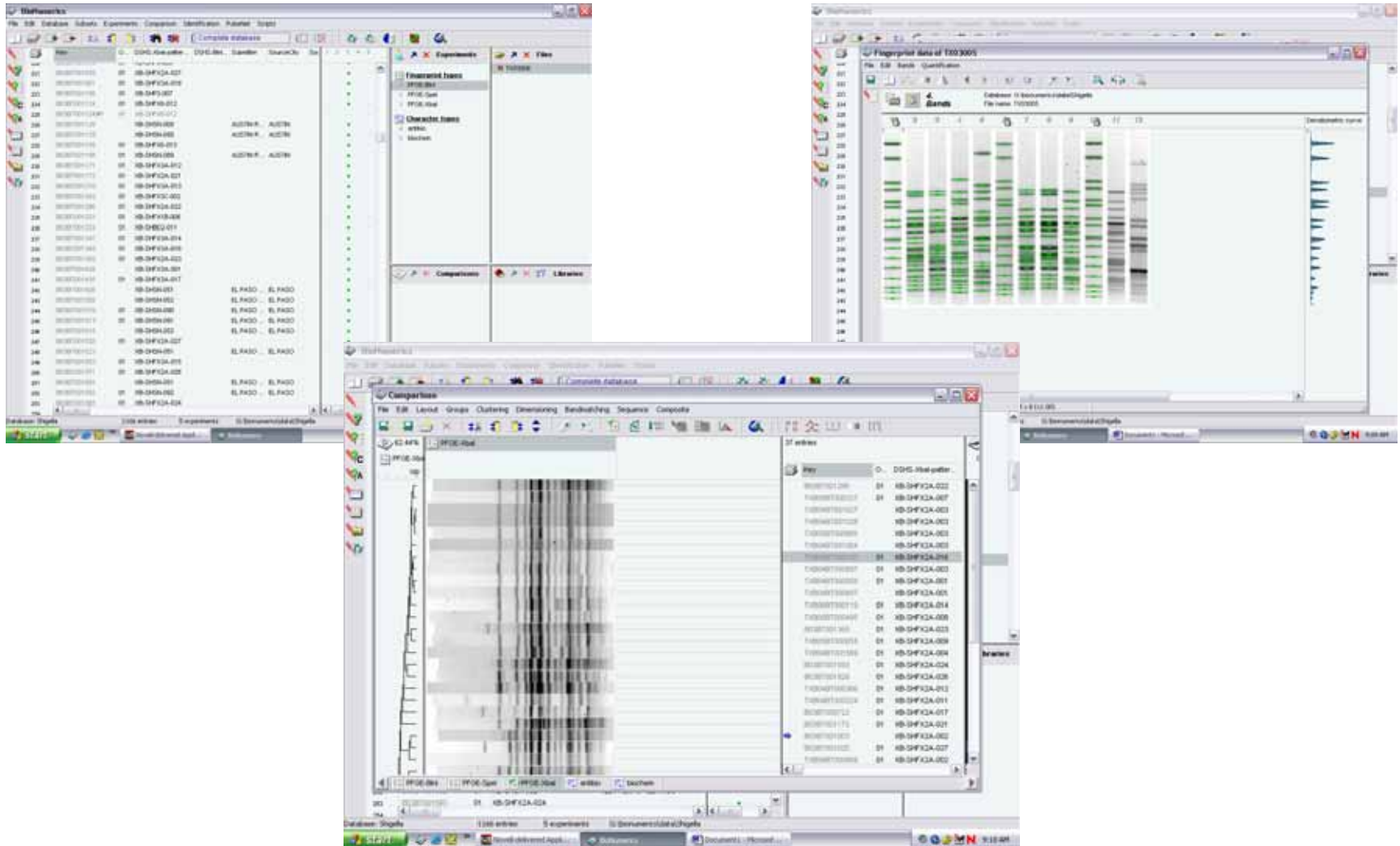
Gel imaging system



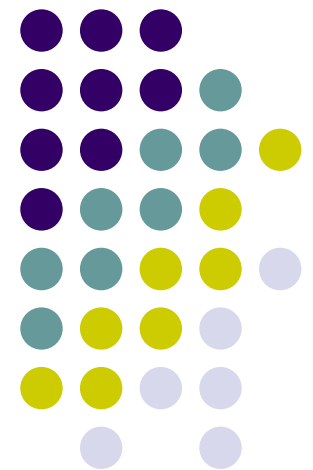
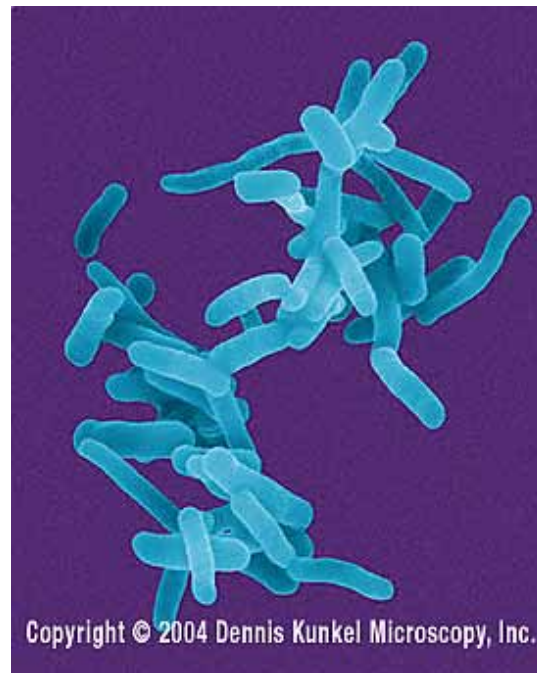
Gel Image



BioNumerics Program



Results

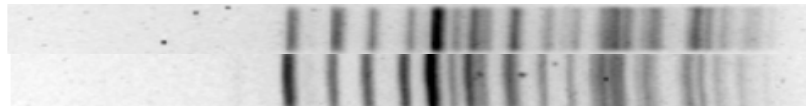
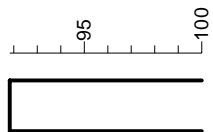


ATCC Specimens (42)

- *S. boydii* (17), *S. dysenteriae* (12), *S. flexneri* (11), and *S. sonnei* (2)
- All strains were compared with the entire TDSHS database (>1000)
- One ATCC *S. flexneri* 2B strain matched with a *S. flexneri* 2B clinical isolate in the database

2003 Clinical Isolates

- *S. flexneri* (66) and *S. boydii* (7)
 1. Compared with other 2003 isolates
 - a. 2 *S. flexneri* 2A isolates from different patients matched each other



B03BT000790

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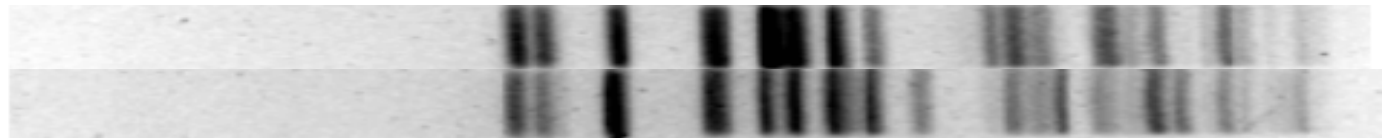
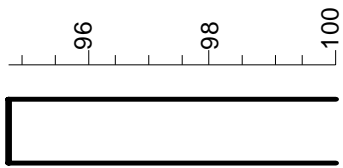
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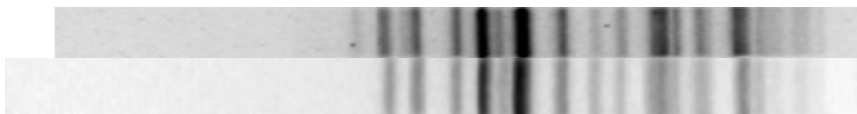
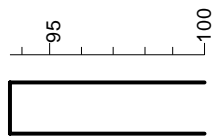
2003 Clinical Isolates

- b. 2 *S. flexneri* Y variant isolates from the same patient did not match with each other



2003 Clinical Isolates

2. Compared with the TDSHS database
 - a. One *S. flexneri* 2A isolate matched a *S. flexneri* 2A clinical specimen from 2004



B03BT001003

XB-SHFX2A-002

TXB04BT000869

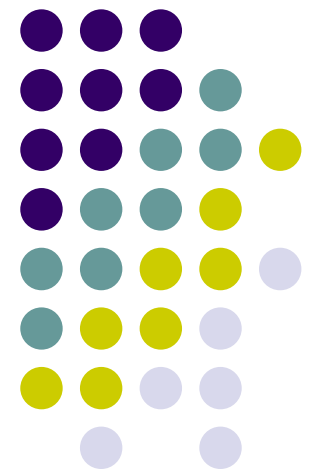
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XB-SHFX2A-002

Conclusion and Future Studies



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Additions to the TDSHS Database

- ▣ Added 42 ATCC specimens and 73 clinical isolates from 2003 to the TDSHS database
- ▣ New additions to the database increase the power of epidemiologists to compare and identify increases in prevalence of a certain strain

Observations

- *S. dysenteriae* and *S. boydii* banding patterns for each serotype very distinct
- *S. flexneri* isolates showed much higher number of bands in the lower half of the gel
 - Harder to differentiate
 - Poorer resolution
 - Many more new patterns identified because of intricate differences

Matching *S. flexneri* Specimens

- ❑ One 2B ATCC specimen matched a 2B isolate in the database
- ❑ One 2A clinical isolate from 2003 matched an isolate in the database
- ❑ One 3A clinical isolate from 2003 matched 5 other isolates in database
- ❑ Two 2A clinical isolates from different patients in 2003 matched each other

Why so few matches?

□ ATCC

- Few matches were expected—only one found
- From national culture library
- Commonly seen strain of *Shigella flexneri* 2A

□ 2003

- TDSHS receives only a fraction of samples from all over state
- Most did not match each other or others in database
- Those that did matched each other—unrelated

Two Interesting Findings

- 2003-two different strains of *S. flexneri* Y variant were isolated from the same patient
 - The genotypes were similar but not identical
 - Genetic mutation cause change in banding pattern

- 2004-two different patients infected with same strain of *S. flexneri* 3A
 - Demographic information of two of the patients showed that they lived in neighboring cities
 - These two cases may be related

Future Studies

- Use of different restriction enzyme(s) to increase resolution of banding patterns for *S. flexneri*
- Epidemiology study
- Collect samples from known outbreaks

Acknowledgments

- ❑ Ana Maria Valle-Rivera, Ph.D.
- ❑ Eric Casey and other members of the Molecular Biology Laboratory
- ❑ Leanne Field, Ph.D.



Special Thanks to:

- The Centers for Disease Control and Prevention, Epidemiology and Laboratory Capacity for Infectious Diseases Program