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

1. Prepare cultures
2. Harvest bacteria
3. Encase bacteria in agarose
4. Digest cellular components
5. Wash agarose plugs
6. Digest DNA with restriction enzyme
7. Cast gel
8. Perform PFGE
9. Stain gel
10. Destain gel
11. Photograph gel
12. Analyze profile
13. Compare banding patterns
Washing agarose plugs
Agarose Gel Mold
Electrophoresis system
Gel imaging system
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
### ATCC match

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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
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
a. One *S. flexneri* 3A isolate matched 5 other *S. flexneri* 3A isolates from 2004 and 2006.
Conclusion and Future Studies
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
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