Alison Weiss, PhD
Professor Molecular Genetics

Shiga Toxin –
Genes on the Move
Outline

- Case Reports - O104:H4 Outbreak
- Diarrheagenic E. coli
- Shiga toxin - Hemolytic Uremic Syndrome
- Shiga toxin - Genes on the move
- The Antibiotic Connection

Going Forward
May 17, 2011, a 16-year-old girl was admitted to the pediatric emergency ward with bloody diarrhea and abdominal pain. Her laboratory values were normal.
May 17, 2011, a 16-year-old girl was admitted to the pediatric emergency ward with bloody diarrhea and abdominal pain. Her laboratory values were normal.

Later that day, her 12-year-old brother was admitted. He had a 2-day history of malaise and headache and a 1-day history of vomiting and nonbloody diarrhea.

Presented with acute renal failure, fulfilled the case definition for hemolytic uremic syndrome

- Serum creatinine level, 4.1 mg per deciliter
- Potassium level, 6 mmol per liter
- Thrombocytopenia (22,000 platelets per cubic millimeter)
- Hemolytic anemia (hemoglobin, 11.6 g per deciliter)
- Bilirubin, 2.8 mg per deciliter
- Lactate dehydrogenase, 2297 U per liter.

Hemoglobin level fell to 8.4 g per deciliter within 48 hours
A week earlier the family meal included a freshly prepared salad containing bean sprouts. The mother remained well, the father developed hemolytic uremic syndrome.

**Stool samples:**
- Plated on Sorbitol-MacConkey agar
- Liquid enrichment culture

**Results:**
- Liquid cultures - positive for Shiga toxin by ELISA
- Bacteriology: Sorbitol positive (therefore NOT O157:H7)
- PCR positive for \( stx2 \) gene, negative for the \( stx1 \) and \( eae \) genes (therefore NOT O157:H7)
- Not reactive with serum against the most common types of Shiga toxin \( E. coli \) (therefore NOT O157:H7)

**Rare serotype O104:H4,**
- harboring the extended spectrum beta-lactamase gene
The 16-year-old girl had a mild course of disease, did not develop HUS, and was discharged from the hospital on the same day.
The clinical picture for her 12-year-old brother was much less benign.

- Renal function, hemoglobin level, and thrombocytopenia improved after 9 days of peritoneal dialysis

- He developed severe neurologic symptoms including: somnolence, visual impairment, speech disturbances, hemiplegia, and incontinence

- He underwent four cycles of plasmapheresis and therapy with the anti-C5-antibody eculizumab.

- After this treatment, his clinical condition improved.

He was discharged after 24 days with serum creatinine levels just above the normal range.

However, he was left with neurologic sequelae and required rehabilitation.
Unusual Features of German Outbreak

Rare serotype of Shiga toxin producing *E. coli*, previously only isolated twice from sporadic cases of hemolytic uremic syndrome

Unusual presentation of hemolytic uremic syndrome:
- Developed in about 25% of cases, versus 1-15% in previous outbreaks
- Most cases in adults, instead of children
- More common in females (68%) than males

Longer incubation period (7-12 days)

No zoonotic source
Lessons Learned

Diagnosis was hampered by use of laboratory tests designed to detect strains previously associated with hemolytic uremic syndrome (O157:H7)

Instead – Identify Shiga toxin producing *E. coli*

Bacteriologic investigation ineffective, >10,000 food samples, all tested negative

**Tracing back:**
Identified common foods and supply chains

**Tracing forward:**
Identified clusters supplied by sprout producer
July 26 - Germany’s Federal Disease Control Declared Epidemic Over

Overall 4,400 infected
> 800 cases hemolytic uremic syndrome, 51 deaths
Two Clusters - Largest in Northern Germany
Smaller cluster - France
US - 5 imported cases - one death

Produce wars:
• Spain (innocent victim - cucumbers misidentified as source)
• Russia (the heavy - stopped all produce imports)
• Egyptian fenugreek source of outbreak, European Union placed temporary ban on all seeds and beans from Egypt
• Cairo denied responsibility, said contamination occurred during re-packing or the water used for sprouting

Produce growers -
Promised 227 million Euros in compensation
Role of DNA Sequencing

Open-source genomics was used to investigate the origin and pathogenic potential of the outbreak strain.

High-throughput sequencing generated genome sequences within days.

Public data release allowed for rapid analysis by bioinformaticians worldwide.
Outline

- Case Reports - O104:H4 Outbreak
- Diarrheagenic *E. coli*
- Shiga toxin - Hemolytic Uremic Syndrome
- Shiga toxin - Genes on the move
- The Antibiotic Connection

Going Forward
Most *E. coli* harmless, some highly pathogenic

Mobile Genetic Elements Promote Evolution to Virulence

Pathogenic E. coli

Transposon
Pathogenicity Island
Phage
Plasmid

Commensal

Dysentery
Diarrhea

Meningitis
Urinary Tract Infection
Hemolytic Uremic Syndrome
Harmless *E. coli* -
Only two traits needed to Become Diarrheagenic

1. *E. coli* must be able to adhere to cells of the intestinal tract

2. *E. coli* must be able to disrupt intestinal tract function

*E. coli* has several different genetic programs to become a diarrheagenic pathogen
Genetic Relationships between *E. coli* Pathotypes

Diarrheagenic - Enteropathogenic *E. coli*

1. **EPEC** - Attach to small bowel (bundle-forming pili)
2. Damage intestinal tract - Protein translocated into cytoplasm induce cytoskeletal changes which destroy the normal microvillar architecture (attaching and effacing lesions)
   - Leads to an inflammatory response and diarrhea.
1. **EHEC** - Attach to colon
2. Damage intestinal tract - Protein translocated into cytoplasm induce cytoskeletal changes which destroy the normal microvillar architecture (attaching and effacing lesions)

- Produce **Shiga toxin** - Life threatening, systemic complications
Evolution to Virulence

Evolution from EPEC (Pathogenic) to EHEC (Hemorrhagic) via Phage and Shiga toxin.

EPEC (Pathogenic) + Phage = EHEC (Hemorrhagic)

Shiga toxin

E. coli O157:H7

Diarrheagenic → Deadly
1. EAEC adheres to small and large bowel epithelia in a thick biofilm

2. Produce toxins which promote diarrhea and damage intestinal tract
Diarrheagenic Enteroaggregative *E. coli*

EAEC (Aggregative)
Evolution to Virulence

Diarrheagenic $\rightarrow$ Deadly

German Outbreak Strain O104:H4
Outline

- Case Reports - O104:H4 Outbreak
- Diarrheagenic *E. coli*
- **Shiga toxin** – Hemolytic Uremic Syndrome
- Shiga toxin – Genes on the move
- The Antibiotic Connection

Going Forward
Shiga Toxin

AB$_5$ toxin

A - active subunit, RNA N-glycosidase
Cleaves ribosomal RNA
Activity, halts protein synthesis
Causes cellular death

B - binding subunit, binds glycolipid, Gb3
Shiga Toxin

Two forms, Stx1 and Stx2, share about 60% amino acid identity

Stx2 (LD$_{50}$ mice = 6 ng) is more potent than Stx1 (LD$_{50}$ mice = 1000ng)

Stx2 but not Stx1 is associated with Hemolytic uremic syndrome

Hemolytic Uremic Syndrome

Characterized by hemolytic anemia, low platelet count (thrombocytopenia) and acute renal failure (uremia)

Resulting from activation of clotting cascade and direct (or indirect) damage to the kidney
Shiga Toxin

Molecular Basis for Shiga toxin-mediated Hemolytic uremic syndrome Is not well understood

May require two assaults on the Circulatory system

1. B-pentamer activates clotting cascade
2. Protein synthesis inhibition damages kidney and/or activates inflammatory responses
Shiga Toxin and Hemolytic Uremic Syndrome

Stx B-pentamer promotes release of Von Willebrand Factor, initiating clotting cascade
Shiga Toxin and Hemolytic Uremic Syndrome

Protein Synthesis Inhibition: Stress Responses / Cellular Death

- Elevates levels of circulating Pro-inflammatory cytokines (IL-6, IL-8) and Anti-inflammatory cytokines (IL-10, IL-1 receptor antagonist)

Renal proximal tubular epithelial cells are extremely sensitive to Shiga toxin
Outline

- Case Reports - O104:H4 Outbreak
- Diarrheagenic *E. coli*
- Shiga toxin - Hemolytic Uremic Syndrome
- **Shiga toxin - Genes on the move**
- The Antibiotic Connection

Going Forward
Shiga Toxin is Phage Encoded

Phage Life Cycle

Lytic Infection
Preferred Pathway
Viral Replication
Death of *E. coli* host

Lytic cycle activated by stress

Lysogeny
DNA integrated into *E. coli* genome
All genes silent, Except repressor

Repressor

Host DNA integrated into *E. coli* genome
All genes silent, Except repressor

Lytic cycle activated by stress
Viral Late Genes
Genome replication, Heads, Tails, Bacterial Lysis and SHIGA TOXIN!!!

Shiga toxin genes are silent during lysogeny

STEC
Repressor
SOS stress response results in proteolysis of the repressor

Stress!!!! ($H_2O_2$ neutrophils, Antibiotics)
Phage and Shiga toxin are produced and released by cell lysis.
Shiga toxin is only made when the bacteria are going to die from the phage lytic cycle.

What is the selective advantage of Lysogeny?

Why link toxin production to the Lytic cycle?
Lysogeny

Maintained by phage repressor, confers resistance to the same immunity type
Lysogeny confers a competitive Advantage in mixed populations

Lysogens resistant to infection by phage

Phage infection usually kills non-lysogenic *E. coli*
Why link toxin production to the Lytic cycle?

- **Confers a survival advantage**

Shiga toxin can kill eukaryotic predators such as *tetrahymena*
Why link toxin production to the Lytic cycle?

- Provides for toxin secretion

Suicide toxin secretion – Phage lysis mediates secretion.

No need for Type 2 or Type 4 secretion
Why link toxin production to the Lytic cycle?

• Genetic expansion
Hypothesis:
Intestinal \textit{E. coli} infected with the Shiga toxin-encoding phage could produce Shiga toxin.

- **Susceptible Host \textit{E. coli}**: Infected with \textit{O157}, lysis, basal levels of toxin released. Amplification of toxin and virus.

- **Resistant Host \textit{E. coli}**: Infected with \textit{O157}, lysis, cannot infect host \textit{E. coli}. Only basal toxin levels released.
In vitro method to assess the influence of non-pathogenic E. coli on Shiga toxin production

Incubate intestinal E. coli with O157:H7 phage

Measure Stx using Vero cells

O157:H7 supernatant
Infection of intestinal *E. coli* can result in toxin amplification.

Does this occur in vivo?  
Mouse Model of Infection

Timeline

<table>
<thead>
<tr>
<th>day</th>
<th>-9</th>
<th>-7</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>Fed Human E. coli Phage Resistant or Sensitive (10⁹ cfu)</td>
<td>Challenge with clinical isolate of O157:H7 (10⁶ cfu)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Collect feces to determine
  - colonization
  - toxin production

- No toxin was ever recovered from the mice colonized with the phage-resistant strain.
High levels of Shiga toxin were recovered from some mice colonized with the phage-sensitive strain.

- 9/9  6/9  6/6  3/6  3/3  2/3

**Toxin Recovery in Feces**

<table>
<thead>
<tr>
<th>Fecal Toxin (ng/g)</th>
<th>Resistant Ec + O157</th>
<th>Sensitive Ec + O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>above LOD</td>
<td>below LOD</td>
<td></td>
</tr>
</tbody>
</table>

- Limit of Detection
- below LOD/total mice
Why link toxin production to the Lytic cycle?

- Primarily produced by doomed bugs

  \[ \text{e.g. H}_2\text{O}_2 \text{ from neutrophils} \]
Why link toxin production to the Lytic cycle?

- Primarily produced by doomed bugs

  e.g. antibiotics
Outline

• Case Reports – O104:H4 Outbreak
• Diarrheagenic *E. coli*
• Shiga toxin – Hemolytic Uremic Syndrome
• Shiga toxin – Genes on the move
• The Antibiotic Connection

Going Forward
Ciprofloxacin Increases Shiga Toxin Expression

Antibiotics and Shiga Toxin Production

Antibiotics with Therapeutic Potential:

**Translation:**
- Azithromicin
- Gentamicin
- Doxycyclin

**Cell Wall:**
- Ampicillin
- Ceftriaxone

**Transcription:** Rifampicin

**Contraindicated - Target DNA Synthesis**
- DNA gyrase: Ciprofloxacin
- Purine synthesis: Trimethoprim/sulfamethoxazole
Summary:
Phage encoded Shiga toxin confers a selective and fitness advantage to lysogenic strains.

Antibiotics:

**Azithromycin** – shows promise

Trimethoprim/sulfamethoxazole, Ciprofloxacin

- Increase Shiga toxin production in GI tract leading to more serious disease
- Could increase transmission of the Shiga toxin encoding phage – leading to evolution of more serious pathogens
Summary:

New pathogenic forms of *E. coli* Can emerge at anytime

Outbreak investigations severely hampered by looking for the

“Usual Suspects”
Pathogenic *E. coli*

Transposon | Pathogenicity Island | Phage | Plasmid
---|---|---|---

Commensal

Dysentery | Diarrhea
---|---

Hemolytic Uremic Syndrome

Meningitis | Urinary Tract Infection
---|---

Nature Reviews | Microbiology
Shiga Toxin
Treatment Options In the Pipeline:

- Anti-toxin antibodies
- Toxin neutralizers (receptor mimics)
- Anti-complement C5-antibody eculizumab, showed some promise

Eliminate circulating toxin, but cannot reverse toxin-mediated damage.
Prevention – irradiation of food

Develop Shiga toxin toxoid vaccine
Acknowledgements

NIAID:
RO1 AI064893
U01 AI075498
T-32 Biothreat Agents Training Grant

Albert J. Ryan Foundation

Consortium for Functional Glycomics

Digestive Heath Center
Cincinnati Children’s Hospital Research Foundation
Acknowledgments

- **Weiss Lab (past and present)**
  - Karen Gallegos
  - Colleen McGannon
  - Shantini Gamage
  - Cindy Fuller
  - Sayali Karve
  - Christine Pellino
  - Charles Talbott
  - Mike Flagler
  - Kayleigh MacMaster
  - Scott Millen
  - Thusitha Gunasekera

- **Jane Strasser Lab**
  - Claudia Chalk

- **Andrew Herr Lab**
  - Deb Conrady

- **Suri Iyer Lab**
  - Sujit Mahajan
  - Ashish Kulkarni
  - Dan Lewallen

- **Rhett Kovall Lab**
  - Dave Friedmann
  - Brad VanderWielen