Shiga Toxin

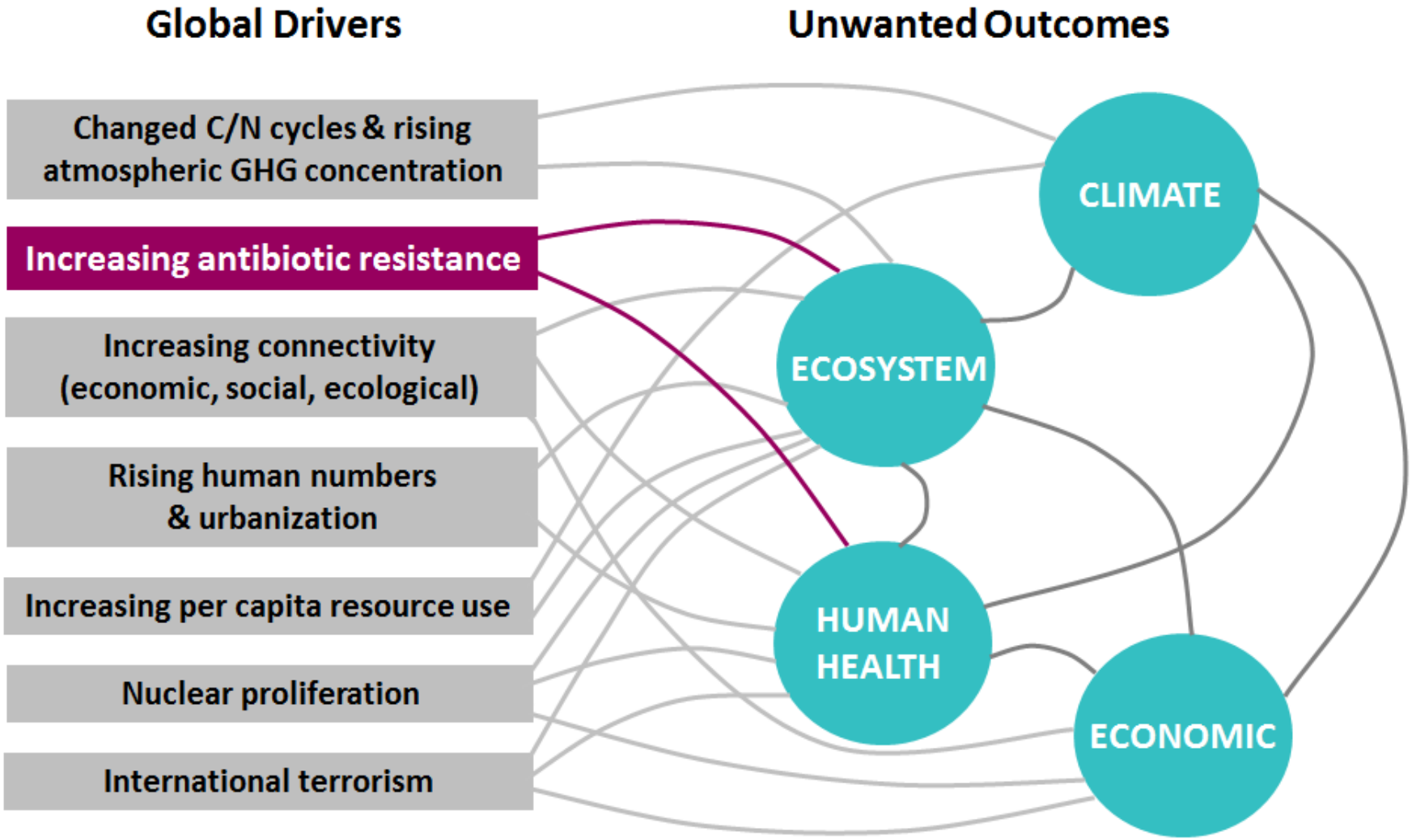
Norman Moore, PhD

Director of Scientific Affairs, Infectious Diseases, Alere

Objectives

- Discuss the clinical significance of Shiga-toxin producing *E. coli* (STEC) and its effect on public health
- Demonstrate how STEC can be transmitted and what foods are at highest risk
- Review the ways in which STEC causes significant illness, especially in younger children
- Analyze the need for and how to test and treat for STEC

Top 7 Threats to the Human Race



Source adapted from Science, Vol 325, September 2009
Available at http://www.sciencemag.org/content/325/5948.cover-expansion

Infectious Disease & Antibiotics

1970: Surgeon General William Stewart said the US was “ready to close the book on infectious disease as a major health threat”

- Modern antibiotics, vaccination, and sanitation methods had done the job

1995: Infectious disease is the 3rd leading cause of death behind heart disease & cancer

2015: Infectious disease remains a critical concern as antimicrobial resistance increases

Antibiotic Overuse

One in every three inpatients will receive 2 or more antibiotics in the course of their hospital stay

Of the inpatients receiving antibiotics, 3/4 will receive unnecessary or redundant therapy

Each year, **tens of millions** of antibiotics are prescribed unnecessarily for viral upper respiratory infections

CDC – Get Smart Campaign

Costs of Antibiotic Resistance

Antibiotic resistance increases the economic burden on the entire US healthcare system

- Resistant infections cost more to treat and can prolong healthcare use

More than **$1.1 billion** is spent annually on **unnecessary** antibiotic prescriptions for respiratory infections in adults

In total, antibiotic resistance is responsible for:

- $20 billion in excess healthcare costs
- $35 billion in societal costs
- 8 million additional hospital days

CDC – Get Smart Campaign

“A post-antibiotic era means, in effect, an end to modern medicine as we know it. Things as common as strep throat or a child’s scratched knee could once again kill.”

Margaret Chan, WHO Director General

Why Antimicrobial Stewardship?

- A balance of infection prevention and antibiotic management

✓ Achieve optimal clinical outcomes

✓ Decrease adverse drug events
  - *C. difficile*

✓ Minimize development of antimicrobial resistance

✓ Preserve antimicrobial resources

✓ Reduce costs

Antimicrobial Stewardship Programs

- Guidelines for Developing an Institutional Program to Enhance Antimicrobial Stewardship – 2006
  http://www.idsociety.org

- Core members include:
  - Infectious Disease Physician
  - Clinical Pharmacist
  - Clinical Microbiologist
  - Infection Control Professional
  - Information System Specialist

Rapid Diagnostics

Test, Target, Treat

- Know the organism, know the appropriate treatment

Reduce antibiotic overuse & unwanted side effects

Shorten time to appropriate therapy

Provide targeted treatment with narrow-spectrum agents when possible

Reduced infection transmission increases infection prevention savings

Gastrointestinal Disease: Impossible but True

- Impossible to diagnose on clinical symptoms alone, but frequently done
- What's the primary symptom of any GI disease?

- 100s of causes, often treated empirically with antibiotics

Diarrhea

Worldwide, it kills 1.5 to 2.5 million children per year

- 5 to 18 cases/year

US statistics

- Adults average 1 acute case/year
- Young children 2 acute cases/year

Diarrhea in the US

211-375 million diarrheal illnesses/year

- 21-37 million episodes in children under five

73 million physician consultations

1.8 million hospitalizations

3100 deaths

$25 billion in healthcare costs

Foodborne Illness in the US

76 million illnesses

325,000 hospitalizations

5000 deaths

IDSA Guidelines 2001

Evaluate severity and duration
Obtain history and physical examination[1-5]
Treat dehydration
Report suspected outbreaks[6]
Check all that apply:[7]

A. Community acquired or traveler's diarrhea

(esp. if accompanied by significant fever or blood in stool)

Culture or test for:
Salmonella
Shigella
Campylobacter
***E. coli* O157:H7** (if blood in stool also test for Shiga toxin and refer isolates if toxin pos.)
***C. difficile* toxins A ± B** (if antibiotics or chemotherapy taken in recent weeks)

Consider quinolone for suspected shigellosis in adults (fever, inflammation); macrolide for suspected resistant *Campylobacter;* avoid antimotility or certain antimicrobial drugs if suspected STEC (afebrile, bloody diarrhea)[8]

B. Nosocomial diarrhea

(onset after >3 d in hospital)

Test for
***C. difficile* toxins A ± B**
(In suspect nosocomial outbreaks, in patients with bloody stools, and in infants, also add tests in panel A)

Discontinue antimicrobials if possible; consider metronidazole if illness worsens or persists

C. Persistent diarrhea >7d

(esp. if immunocompromised)

Consider parasites[9]
Giardia
Cryptosporidium
Cyclospora
Isospora belli

+ Inflammatory screen[7]

☐ **If HIV pos., add:**

Microsporidia
(Gram-chromotrope)
***M. avium* complex**
+ panel A

Treat per results of tests

Etiological Agents of Diarrhea

Viral infections

- Rotavirus, Norovirus, Cytomegalovirus (in immune compromised), Astrovirus, Adenovirus, etc.

Bacterial infections

- *Campylobacter*, *Salmonella*, *Shigella*, and *E. coli*

Parasites

- *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*

Escherichia coli (E. coli) Basic Information

Ubiquitous in the gut of healthy individuals & many animals

- Most strains are not only harmless, but beneficial

May cause illness

- Diarrhea
- Urinary tract infections
- Respiratory illness & pneumonia

Can be used to monitor water quality

- Fecal coliforms are bacteria that live in stool of warm-blooded animals
- Can have direct contamination of water with stool
- Can have run-off from farm irrigation
- Can have breaks in septic systems

What are Shiga-Toxin Producing *E. coli*?

E. coli that can produce Shiga toxin (STEC)

- Also called Entererohermorrhagic *E. coli* (EHEC) and verocytotoxic *E. coli* (VTEC)
- Identical toxin to what is produced in *Shigella*

Most famous is *E. coli* O157:H7

- Other non-O157 STEC serogroups that often cause illness in people in the United States include O26, O111, and O103.

Shiga toxin

- Comes in two main groups; Stx1 and Stx2
- Toxin that inhibits protein synthesis in target cells
- Stx2 more associated with complications like HUS

Additional Issues with STEC

Bacteria are often acid tolerant

Low infectious dose (<100 organisms)

More common during the summer months

What are the Symptoms of STEC?

Varies per person

- Many can be subclinical

Main symptoms

- Diarrhea (potentially bloody)
- Severe stomach cramps
- Vomiting
- May have fever

Disease Resolution

- Usually 5 to 7 days

Complications of STEC

Of those diagnosed with STEC, around 5-10% can get hemolytic uremic syndrome (HUS)

Symptoms of HUS

- Bloody diarrhea
- Decreased urination
- Tired
- Loss of pink color in cheeks and inside of lower eyelid

Major Issue of HUS

- Kidneys may stop working among other complications
- People with HUS may recover in weeks, suffer permanent damage, or potentially death

How Do Patients Develop HUS?

Mechanism

- The toxin causes the blood cells to become misshapen
- Blood cells can clog the tiny vesicles in the kidneys known as glomeruli
- Filtration by the kidneys becomes blocked
- Kidneys may shut down

Usually children

Thrombotic Thrombocytopenic Purpura (TTP)

Mechanism

- Toxin changes shape of the blood cells
- Clots form in blood vessels
- Oxygen does not get to organs like brain, heart, and kidneys
- Platelets get used up
- Bleeding problems arise whether internally, under skin, or cut

More likely in adults

Incubation Period

Average time between consumption of STEC to disease is 3-4 days

- Time can vary from 1 to 10 days

Symptoms increase over time

- Stomach ache to mild diarrhea
- Increases to more significant diarrhea to bloody diarrhea

HUS, if it happens, usually is around a week after initial symptoms

STEC Stats

CDC estimates 265,000 cases per year in the US

- *E. coli* O157:H7 accounts for roughly 36% of these infections

Why aren’t they seen in hospitals?

- Many patients don’t seek medical attention
- Those patients that do may not give a stool sample
- Laboratories may not be able to test stool samples

Infecting Others

Typically, toxin-producing bacteria go away with symptoms

For some individuals, symptoms may resolve, but can still have STEC for months

Therefore, important to keep up appropriate handwashing!


E. COLI
OUTBREAK

Transmission of STEC

Fecal oral route

- Directly from people not washing hands and touching others
- Indirectly from people not washing hands and handling food
- Daycares with diapers

Food exposure

- Meat can be directly contaminated during processing
- Other foods like water runoff from cow pasture to farm

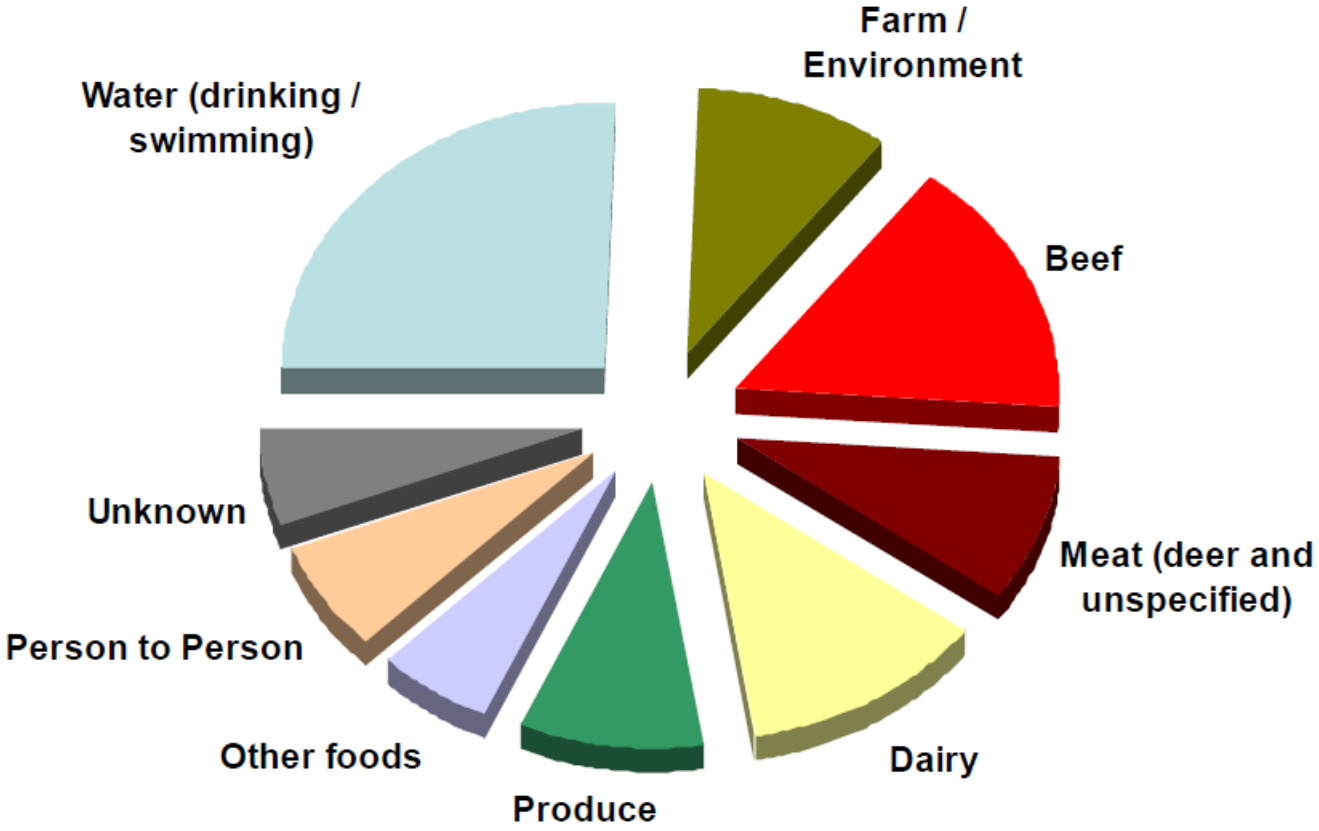
Environmental Exposure

- Lake/pool/water park
- Petting zoos & other animal exhibits

STEC Transmission

- Community acquired
- Food & water borne
- Outbreaks

E. coli O157:H7 Outbreaks Worldwide, 1982–present



Printed with permission from Ellin Doyle. From: Human Illness Caused by E. coli O157:H7 from Food and Non-food Sources. http://fri.wisc.edu/docs/pdf/FRIBrief_EcoliO157H7humanillness.pdf

Multistate *E. coli* O157:H7 in Ground Beef – June 2014

Major statistics

- 12 cases with 7 being hospitalized
- Spanning 4 states
- Zero deaths

Why

- Contaminated ground beef at Wolverine Packing company
- 1.8 million pounds distributed to restaurants and retail throughout the nation

Action

- Recall happened AFTER use-by date
- Consumers should check their frozen meat

Multistate *E. coli* O121 in Raw Clover Sprouts – August 2014

Major Statistics

- 19 cases with 44% hospitalization
- 6 states
- Zero deaths

Why?

- Evergreen Fresh Sprouts, LLC of Idaho had contaminated raw clover sprouts
- In interviews, 81% of people ate raw clover sprouts the week before, often from local restaurants

Action

- FDA inspection saw multiple unsanitary conditions often involving corroded metal in contact with food
- The suspect seed lot was discontinued

Multistate *E. coli* O121 in Farm Rich Brand Frozen Food – May 2013

Major Statistics

- 35 cases with 9 hospitalizations
- 19 states
- Zero deaths, but 82% were under 21 and 2 developed HUS

Why?

- O121 found in frozen mini pizza slices

Action

- Recall expanded to include all Farm Rich, Market Day, and Schwan's brand frozen food products

Diagnostic Recommendations

Why Should People Test?

Hydration

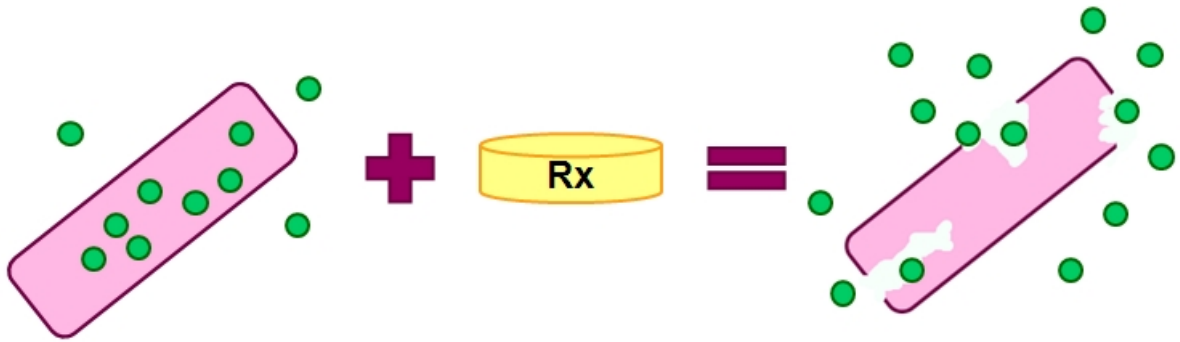
- May want to treat with parenteral volume expansion if it is early in the infection

Do not take antibiotics

- Increases risk of HUS
- Antidiarrheal agents like Imodium may also increase risk of HUS

Antibiotics and STEC

- Potentially fatal renal failure
- More prevalent in pediatrics
- Antibiotics may increase risk of HUS due to toxin release from dying STEC cells



Who should be tested?

- CDC & Joint Commission: All acute community acquired diarrheas

Screening Criteria	Test if	Risk
Visible blood	Present	Not all STEC positives have visible blood
Season	Summer-Fall	Can occur year-round, esp. with imported produce
Patient Age	Pediatrics, Elderly	Not all cases are peds & elderly

- Antibiotic use is common for GI disease, may be deadly in a STEC patient
- Rapid identification of potential outbreaks

O157 and non-O157 strains

O157:H7 is the most common US strain

- Traditionally labs only tested for O157

Over 100 non-O157 strains cause disease

- April 2013 outbreak of O121
- 2011 German outbreak of O104
  - Over 3,500 cases, 23% developed HUS

Detecting O157 vs. non-O157

O157-specific Assays
Test for O157 strain only
Will miss all non-O157 STEC strains
Plate culture (SMAC, mac-sorb) most common

Assays for Toxins
Test for toxins Stx1 & Stx2
Will detect all toxin-producing strains
Rapid assay or ELISA most common (may require broth enrichment)

CDC Recommendations

Test all stools from community-acquired diarrhea for STEC

Concurrent testing

- Culture stool for O157
- Testing for Shiga toxin

Actions

- All O157 STEC isolates should be forwarded to appropriate public health lab for confirmatory testing & additional characterization
- Results should be reported promptly to physician

Rationale for CDC Recommendation

There are publications supporting that STEC may be as common as other bacterial pathogens

- STEC (0% - 4.1%)
- *Salmonella* (1.9% - 4.8%)
- *Campylobacte*r (0.9% - 9.3%)

Early diagnosis is important for proper treatment

- Volume expansion for O157
- No antibiotics to lessen risk of HUS

Help detect outbreaks earlier

Why Every CA Stool?

Many infections can be missed when having selective strategies

- Just children
- Just summer

Some STEC don’t have visible blood and other pathogens may

Pathogens will be more difficult to recover from stools at a later date

Help prevent transmission to others

- Children may not be able to return to daycare facilities
- Food service workers

E. coli O157 Culture

Plate on sorbitol-MacConkey agar plate or chromogenic agar

- O157 does not ferment sorbitol within 24 hours

Wait 16-24 hours @ 37°C

Look for color

- SMAC plates have colorless colonies
- Chromogenic agar has specific colony colors

Test well-isolated colony on antiserum or latex

- Screen at least 3 colonies

Preliminary findings reported

Confirmatory testing

Shiga Toxin Assays

Major benefit

- Detects all Shiga-toxin producing *E. coli* rather than just O157

What to test?

- See manufacturer's product inserts on what is approved

Direct stool

- Fresh
- Frozen

Cultures

- Broth culture
- Plate culture

Stool testing For Shiga Toxin

Major Benefit – Detects toxin producers beyond *E. coli* O157

Direct stool detection provides real-time actionable results

- Initiate proper treatment while patient is in the healthcare setting
- Avoid antibiotic exposure

Ideal for emergency situations, pediatrics with bloody diarrhea

Identify potential outbreaks sooner

Molecular Assays

No single Shiga-toxin assay available, but two multiplex assays

Public health laboratories may use home brew molecular

Can be done on isolated colonies or clinical samples

How Does Broth Culture Compare?

2 published studies from Alberta, Canada looked at performance of rapid assays vs. in-house PCR

- “EHEC Assay” study compared market leader in 2013 to PCR
- “STEC Assay” wasn’t available then, study done in 2014
- Similar regional demographics, methods, similar sample size, prevalence rate

Both assays used broth enrichment culture

STEC Assay also tested directly on stool specimens

Chui L, et al. *Diag Micro Infect Dis* 2013. 77(2013): 8-13.

Chui L, et al. *J Clin Micro* e-pub 14 Jan 2015

Broth Culture Comparison

- EHEC Assay not recommended as a routine screening test for STEC from broth cultures due to low sensitivity and risk of false positives

n = 819	Sensitivity	PPV
EHEC Assay (broth culture)	**35%**	**54.5%**

- STEC Assay is a viable alternative to molecular testing for frontline labs as a primary screening method for STEC.

n = 784	Sensitivity	PPV
STEC Assay (broth culture)	**85%**	**100%**

Broth Culture Comparison

Majority of true positives not detected

Almost half of all positive results were false positive

n = 819	Sensitivity	PPV
EHEC Assay (broth culture)	35%	54.5%

n = 784	Sensitivity	PPV
STEC Assay (broth culture)	85%	100%

More positives detected

All positive results were confirmed as true positive

PPV = Positive Predictive Value

Preventing STEC

Hand washing!

- After restroom
- Changing diapers
- Prior to preparing food
- Contact with animals

Prepare food appropriately

- Make sure meat – especially ground beef – is cooked thoroughly
- Don’t use cutting board for meat and then other food
- Clean cutting boards

Avoid drinks that are unpasturized that are supposed to be

- Raw milk
- Dairy products
- Apple cider

Discussion

Shiga toxin producing *E. coli* are a significant health risk

Diagnostic testing can help better direct therapy

Appropriate therapy can reduce complications, such as HUS and TTP