Surveillance of Diarrheagenic *E. coli* Pathotypes

By Sadie Oppegard, MPH, Epidemiology Surveillance Coordinator

Escherichia coli are gram-negative bacteria that are normal flora within the gastrointestinal tract. Strains of *E. coli* can become pathogenic by acquiring certain virulence factors (or mechanisms of disease). Pathogenic *E. coli* can be categorized into six groups known as pathotypes. These include shiga Toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), and Diffusely Adherent *E. coli* (DAEC).

Most clinical laboratories do not routinely test for diarrheagenic *E. coli* other than STEC, as other diarrheagenic *E. coli* pathotypes are not detected by conventional laboratory methods. For this reason, prevalence and significance of these pathotypes in the United States is unknown. However, recently, there has been an increased popularity with laboratories to use multiplex PCR panels. The use of multiplex PCR panels allows for detection of 21 gastrointestinal pathogens, to include the other diarrheagenic *E. coli* pathotypes which were not routinely detected by other methods (such as culture or enzyme-linked immunoassay). One multiplex PCR assay, the BioFire® FilmArray®, detects STEC, as well as EPEC, EAEC, ETEC, and *Shigella*/EIEC targets. With this advancement, public health has begun to assess the clinical significance of the other (non-STEC) diarrheagenic *E. coli* pathotypes in the United States.

STEC has been a priority pathogen in the United States and commonly tested in laboratories. STEC transmission occurs through consumption of contaminated foods, ingestion of contaminated water, or direct contact with infected persons or animals and their environment. DAEC is a relatively new pathotype with little known and no commercially available assays for detection. EIEC is paired with *Shigella* as a target on multiplex PCR panels due the genetic and clinical similarities to shigellosis. EPEC remains an important pathogen of persistent infantile diarrhea in developing countries (along with EAEC), whereas ETEC is a common cause of travelers' diarrhea. Although there is a significant association between these pathotypes and clinical disease (diarrhea), some studies have observed detection in asymptomatic controls. This is especially true for EPEC, which can further be characterized as typical EPEC (tEPEC) and atypical EPEC (aEPEC) by the presence of other virulence factors on the EPEC adherence factor plasmid (pEAF). The presence of the *bfp* gene on the EAF plasmid differentiates tEPEC from aEPEC. tEPEC has been considered a bonafide pathogen, whereas aEPEC has been observed in healthy and asymptomatic controls. Further, other diarrheagenic *E. coli* pathotypes other than STEC are not required by communicable disease regulations to be forwarded to the state public health laboratory for isolation and confirmation of each virulence factor, as funding for testing is limited. These barriers present difficultly with evaluating the clinical significance of these pathotypes in developed countries.

Multiplex detecting EIEC/*Shigella* are considered as an "Shigellosis" investigation by the Nebraska DHHS epi team. *Shigella* species are biochemically and genotypically similar to EIEC and also have growth characteristics of inactive *E. coli*. Additionally, nucleic acid amplification test systems such as BioFire as well as other systems such as MALDI-TOF, and 16S rRNA gene sequencing are unable to differentiate *Shigella* from some strains of *E. coli*, frequently leading to misidentifications. The ability of phenotypic commercial methods to identify *Shigella* was assessed and found to correctly identify only 50-70% of the time even reported as having "good" confidence values on these automated systems. Antimicrobial susceptibility testing (AST) of *Shigella* ssp for ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolone are

recommended by CLSI due to the widespread concern of antimicrobial resistance[1]. Recovery is essential to perform AST on an automated system. Unfortunately, *Shigella* is fragile in transport and recovery is recommended at the originating laboratory. Therefore, recovery is necessary as soon as multiplex detects as positive. Enrichment broth must be subbed after only 6-8h if Shigella is suspected to avoid overgrowth of normal flora[2]. Enrichment broth can enhance recovery of *Shigella* along with parallel plating on such culture medium as XLD agar which detected *Shigella* at 90%. Once isolated, simple phenotypic testing using motility, indole and ornithine (ODC) can rule out any EIEC or *Shigella sonnei* organisms. Any non-motile, indole negative and ODC negative isolates recovered can be forwarded to NPHL for serotyping if not available in-house. The definitive identification of *Shigella* ssp can be accomplished by using the latex agglutination assay with specific antisera or by whole genome sequencing. All *Shigella* species not *sonnei* should be sent to the NPHL for epidemiological testing purposes.

PATHOTYPE	MECHANISM OF PATHOGENESIS	INCUBATION PERIOD	DURATION OF ILLNESS	TYPICAL CLINICAL SYNDROME
STEC	Large bowel adherence mediated via intimin (or less commonly by other adhesions); Shiga toxin 1, Shiga toxin 2 production; Shiga toxin production is linked to induction of the bacteriophages carrying the Shiga toxin genes; some antibiotics induce these bacteriophages	1–10 days (usually 3–4 days)	Typically, 5–7 days; persistent diarrhea (>14 days) has been reported	Watery diarrhea that progresses (often for STEC O157, less often for non-O157) to bloody diarrhea in 1–3 days; abdominal cramps and tenderness; if fever present, low-grade; hemolytic uremic syndrome complicates approximately 6% of diagnosed STEC O157 infections (15% among children aged <5 years) and 1% of non-O157 STEC infections
EPEC	Small bowel adherence and epithelial cell effacement mediated by intimin	9–12 hours	12 days	Severe acute watery diarrhea; may be persistent; common cause of infant diarrhea in developing countries
ETEC	Small bowel adherence via various adhesions that confer host specificity; heat-stable or heat-labile enterotoxin production	10–72 hours	1–5 days	Acute watery diarrhea, afebrile, occasionally severe
EAEC	Small and large bowel adherence mediated via various adhesions and accessory proteins; enterotoxin and cytotoxin production	8–48 hours	3–14 days; persistent diarrhea (>14 days) has been reported	Watery diarrhea with mucus, occasionally bloody; can cause prolonged or persistent diarrhea in children
EIEC	Mucosal invasion and inflammation of large bowel	10–18 hours	4–7 days	Watery diarrhea that may progress to bloody diarrhea (dysentery-like syndrome), fever; similar to shigellosis
DAEC	Diffuse adherence to epithelial cells	Unknown	Unknown	Watery diarrhea but pathogenicity not conclusively demonstrated

Source: https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/escherichia-coli-diarrheagenic

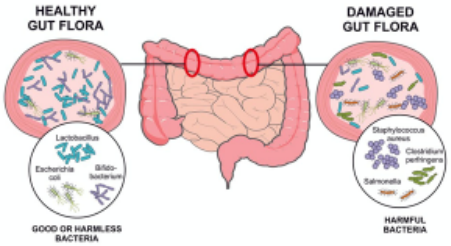
[1] (Buchan, B; *Manual of Clinical Microbiology* 12ed: 688-707)
[2] McElvania, E; *Manual of Clinical Microbiology* 12ed: 132

LHD Presentation from *Sadie Oppegard, MPH, Epidemiology Surveillance Coordinator*

Escherichia coli (STEC) Gastroenteritis: Overview

Disease:

Some kinds of *E. coli* bacteria cause disease when they make a toxin called Shiga toxin. The bacteria that make these toxins are called "Shiga toxin-producing *E. coli*," or STEC for short.



Symptoms:

Diarrhea (often bloody)
Abdominal cramps
Vomiting

Incubation Period:

1- 10 days (average 3-4 days)

Duration of Illness:

5-7 days

Treatment:

Supportive care (fluids and rest)
Antimicrobial therapy for those severely ill – Antibiotics may increase risk of HUS (Hemolytic Uremic Syndromic) in children.

Diarrheagenic *E. coli* Transmission

Fecal-oral route

Contaminated food and water

- Raw or undercooked beef
- Unpasteurized milk or juice
- Raw fruits or vegetables
- Swallowing contaminated recreational water (pool, lake or river)
- Contaminated drinking water (untreated private well, etc.)

Direct animal contact

- Cattle
- Deer
- Horses
- Pigs
- Pets (cats, dogs)

Person-to-person (can be more common due to low-infectious dose)

- Taking care of someone with diarrhea or changing diapers

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EPEC, EAEC, ETEC: Disease Overview

Transmission:
- Contaminated food or water
- Ingestion of untreated recreational water
- Animal contact
- Person-to-person

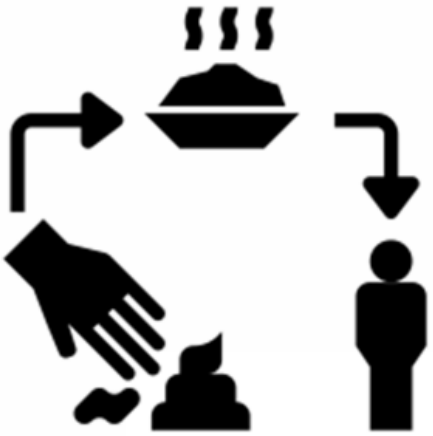
Infectious Dose:
- Generally very low for infants; for adults estimated 10^8–10^{10}

Symptoms:
- Watery diarrhea
- Fever
- Vomiting
- Dehydration

Incubation Period:
- 8 hours – 6 days (average 24 – 48 hours)

Duration of Illness:
- 1-12 days, can be persistent (2-4 weeks)/prolonged (>4 weeks)

Treatment:
- Supportive care (fluids and rest)
- Bactrim, Fluoroquinolones (ciprofloxacin) or macrolides (azithromycin)



Shigellosis: Overview

Disease:

Caused by the bacteria *Shigella*
Four species: *sonnei, flexneri, boydii, and dysentariae*

Symptoms:

Diarrhea (sometimes bloody)
Fever
Abdominal cramps
Nausea and/or vomiting

Incubation Period:

12 hours – 4 days (average 1-3 days)

Duration of Illness:

4-7 days

Treatment:

Supportive care (fluids and rest) –Do NOT use anti-diarrheal medication
Antimicrobial therapy (shortens duration of symptoms and shedding)

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Diarrheagenic *E. coli* Pathotypes

Shiga Toxin-producing *E. coli* (STEC)

- Causes disease from presence of shiga toxin and frequently associated with contaminated products, such as undercooked ground beef and raw produce consumption. Has also been associated with contaminated recreational water and animal contact.

Enteroinvasive *E. coli* (EIEC)

- Genetically and clinically related to *Shigella*, so we consider EIEC a 'shigellosis' investigation.

Diffusely adherent *E. coli* (DAEC)

- New group, little known, not detected by conventional laboratory methods.

Enteropathogenic *E. coli* (EPEC)

EPEC eae gene Stl QI Non-probe PCR:
Detected (qualifier value)

Enteroaggregative *E. coli* (EAEC)

EAEC pAA plas aggR+aatA St Non-probe PCR:
Detected (qualifier value)

Enterotoxigenic *E. coli* (ETEC)

ETEC ltA+st1a+st1b tox St Non-probe PCR:
Detected (qualifier value)

EPEC: Typical (tEPEC) vs. Atypical (aEPEC)

Typical EPEC (tEPEC):

- *eae*+ genes
- *bfp*+ genes
- *Lack stx*- genes

➢ Strongly associated with diarrheal disease

VS

Atypical EPEC (aEPEC):

- *eae*+ genes
- ***Lack bfp*- genes**
- *Lack stx*- genes

➢ Occurrence has been observed in both diarrheal and asymptomatic hosts

No bfp target

EPEC eae gene Stl QI Non-probe PCR:
Detected (qualifier value)

The GI Panel only identifies the *eae* gene and ***does not*** distinguish between tEPEC and aEPEC

The only way to differentiate the two types of EPEC is by isolating the bacteria at the lab and by testing for the presence of both *eae* and *bfp* genes, however we currently do not have the funding to have specimens forwarded to NPHL for isolation and confirmation

Is EPEC is the true etiology for a person with diarrhea and a PCR GI Panel result?

EPEC, EAEC, ETEC: What We Know

- Childhood diarrhea in developing countries

- 'Travelers' Diarrhea'
  (international travel to less-developed countries)
- Not detected by conventional laboratory methods
  (stool culture, enzyme-linked immunosorbent assay)

- Prevalence and significance in the United States is unknown

EPEC, EAEC, ETEC: What We Know

- BioFire® FilmArray® Gastrointestinal Panel is the only multiplex PCR panel able to detect all 3 pathotypes (EPEC, EAEC, ETEC)
- In 2017, Nebraska included the diarrheagenic *E. coli* pathotypes in our rules and regulations for communicable disease reporting
- Several studies have observed detection in both ill and healthy individuals

- Most frequent targets with co-detection of another enteric pathogen

- EPEC, EAEC, ETEC Positives are not submitted to the public health laboratory
- Clinical significance of each pathotype detection requires further study…