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# Investigation of potential Shiga toxin producing *Escherichia coli* (STEC) associated with a local foodborne outbreak using multidisciplinary approaches

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**Abstract**

Shiga toxin producing *Escherichia coli* (STEC) outbreak is a public health concern as it can potentially cause a variety of clinical manifestations including diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). However *E. coli* are generally innocuous commensal organisms, and there is a need to discriminate pathogenic from non-pathogenic isolates rapidly and accurately. In this study, we have used standard culture based methods and advanced molecular approaches to characterize *E. coli* in food in a local outbreak investigation. We show that the application of DNA based detection methods including real-time PCR and DNA microarray along with a traditional culture method can identify the organism implicated in an outbreak at the strain level for pathogenic potential.


*Keywords:* *E. coli* non-O157; Shiga toxin producing *E. coli* (STEC); RT-PCR; DNA microarray

## 1. Introduction

Many serotypes of Shiga toxin producing *Escherichia coli* (STEC) have emerged as a major cause of food-borne infections in the past 30 years [1–3]. *E. coli* serotype O157:H7 STEC is considered one of the most important pathogens for public health concerns, classified as an adulterant by the US Food and Drug Administration and the USDA Food Safety and Inspection Service (FSIS) [4,5]. Recently, it has also been recognized that a large number of *E. coli* non O157:H7 serotypes can be responsible for many *E. coli* outbreaks [6]. A study at the Centers for Disease Control and Prevention (CDC) showed that from 1983 to 2002 approximately 70% of non-O157 STEC infections were caused by strains from one of the six major serogroups known as "Big Six", including O26, O111, O45, O121, O103 and O145 [7]. STEC can potentially cause a variety of clinical manifestations including diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS) [8]. Pathotypically, STEC can usually be classified as enterohemorrhagic *E. coli* (EHEC) [9]. As such, the FDA Bacteriological Analytical Manual (BAM) protocol now requires analysts who perform food testing to screen samples for both *E. coli* O157:H7 and non O157:H7 STEC [8,10].

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For public health concerns, there is a need for rapid methods to identify, characterize and serotype pathogens associated with contaminated food during the surveillance and outbreak investigation. In addition to the traditional culture-based methods, several molecular assays including WGS, real-time (RT) PCR, DNA optical mapping. ELISA or whole genome microarray have emerged over the past several years [11,12]. Our study focuses on using RT-PCR analyses and DNA microarray methods to assist an outbreak investigation of a local *E. coli* non-O157:H7 outbreak causing diarrhea in a few people from a food borne outbreak.

DNA microarray is one of the highly discriminatory sequence-based molecular approaches that can quickly and accurately identify the relatedness of organisms by presence/absence of genes in the pathogens. Low density microarray, targeting several major virulence genes has previously been proposed as a molecular tool to assess STEC [13]. Since then, microarray technology has been improved with the development of bioinformatics as well as whole genome sequencing (WGS) technology [11]. DNA microarray has been shown to provide advantage tools in the characterization of several major foodborne pathogens including *Cronobacter* sp. [14], *E. coli* [11,15–17], *Listeria monocytogenes* [18–20] or *Clostridium botulinum* [21]. We have shown here that after the isolates were obtained, we were able to obtain the genomic information of the isolates within 48 h. The fast turnaround time is critically important not only for outbreak investigation, but is important for the regulatory perspective as well.

## 2. Materials and methods

### 2.1. Sample enrichment and biochemical tests

Our study was carried out in response to a possible local *E. coli* non-O157:H7 STEC outbreak investigation. We used two different approaches to characterize the *E. coli* strains implicated in this outbreak, including culture and molecular based methods. FDA-BAM was strictly followed as a standard method for culturing *E. coli* O157:H7 and *E. coli* non-O157:H7 STEC (BAM chapter 4a) [10]. Several food samples implicated in this outbreak are shown in Table 1. In brief, after the samples were received at our laboratory, microbiological examination was performed within 48 h of the collection, by enriching them in modified buffered peptone water with pyruvate (mBPWp) for 5 h at 37 °C, then supplemented with acriflavin, cefsulodin, and vancomycin (ACV) to the final concentration in mBPWp of 10 mg/L, 10 mg/L and 8 mg/L, respectively. The sample enrichments were then incubated at 42 °C for a total of 18–24 h [10]. The overnight sample enrichments were serially diluted in Butterfield's phosphate buffer, with 0.5 mL of the $10^{-2}$ through $10^{-4}$ dilutions spread-plated in duplicate on Levine-eosine methylene blue (EMB) agar and R&F® non-0157 STEC Chromogenic Plating Medium, a chromogenic agar that utilizes the chromogen, X-β-D-glucuronic acid to detect the enzyme, β-glucuronidase which is produced by 95%–98% of *E. coli*, as well as some *Salmonella* and *Shigella* strains. Addition of phenol red indicator, cellobiose and myo-inositol, differentiates these pathogens from *E. coli*, and after 24 h incubation at 41–42 °C, *E. coli* non-O157 will appear as dark blue colonies with or without a clear ring. Further biochemical testing (MUG, indole) and confirmatory testing such as API20E (BioMérieux, Lyon, France) and Prolex™ *E. coli* non-O157 latex kit (Pro-Lab Diagnostics, Round Rox, TX) were utilized to confirm that both isolates were *E. coli* non-O157 serotype and of the O45 serogroup.

Table 1
Samples that were collected for outbreak investigation.

| Sample ID | Sample type | Microbiological result for *E. coli* |
|---|---|---|
| 001 | Cooked chicken | Negative |
| 002 | Raw chicken | Positive |
| 003 | Sliced almond and raisins | Negative |
| 004 | Grilled salmon | Negative |
| 005 | Better than bouillon 1 | Negative |
| 006 | Better than bouillon 2 | Negative |
| 007 | Curry powder | Negative |
| 008 | Light mayonnaise | Negative |
| 009 | Peeled garlic | Negative |
| 010 | Ginger wasabi sauce | Negative |
| 011 | Soy sauce | Negative |

### 2.2. RT-PCR methods

All sample enrichments were screened for *E. coli* non-O157:H7 STEC using both commercially available BAX-PCR (Dupont, Wilmington, USA) and an in-house real-time PCR [5,22,23]. BAX PCR was performed according to the manufacturer's recommendation. Real-time PCR for non-O157 STEC was performed using ABI7500 FAST Dx with the primers and probes specific to the Big Six group of the *E. coli* non-O157:H7 STEC shown in Tables 2A and 2B.

Primers and probes for RT-PCR (Tables 2A and 2B) obtained from Integrated DNA technologies (Coralville, IA), Biosearch Technologies (Novato, CA) and Invitrogen (Carlsbad, CA) were used to analyze the samples. Big six PCR panel real-time PCR was performed in a final volume of 25 μL in a 96-well plate

Table 2A
Primers sequences for PCR used to screen *E. coli* non-O157:H7 STEC.

| Primer name | Primer sequence |
|---|---|
| 16S RNA F | 5′-CCT CTT GCC ATC GGA TGT G-3′ |
| 16S RNA R | 5′-GGC TGG TCA TCC TCT CAG ACC-3′ |
| wzx O26 F | 5′-GTA TCG CTG AAA TTA GAA GCG C-3′ |
| wzx O26 R | 5′-AGT TGA AAC ACC CGT AAT GGC-3′ |
| wzx O111 F | 5′-TGT TCC AGG TGG TAG GAT TCG-3′ |
| wzx O111 R | 5′-TCA CGA TGT TGA TCA TCT GGG- 3′ |
| wzx O45 F | 5′-CGT TGT GCA TGG TGG CAT-3′ |
| wzx O45 R | 5′-TGG CCA AAC CAA CTA TGA ACT G- 3′ |
| wzx O121 F | 5′-AGG CGC TGT TTG GTC TCT TAG A-3′ |
| wzx O121 R | 5′-GAA CCG AAA TGA TGG GTG CT-3′ |
| wzx O103 F | 5′-TTG GAG CGT TAA CTG GAC CT-3′ |
| wzx O103 R | 5′-ATA TTC GCT ATA TCT TCT TGC GGC-3′ |
| wzx O145 F | 5′-AAA CTG GGA TTG GAC GTG G-3′ |
| wzx O145 R | 5′-CCC AAA ACT TCT AGG CCC G-3′ |