Prevalence of Non- O157 Shiga Toxin-Producing *Escherichia coli* and Enterotoxigenic *Staphylococci* in Ready-to-eat Meat Products, Handlers and Consumers in Cairo, Egypt

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**Abstract:** Shiga toxin-producing *Escherichia coli* and enterotoxin producing *Staphylococci* are potential food borne pathogens and their presence in ready to eat meat products and their handlers indicates that it is necessary to improve hygienic measures during manipulation of meat products. A total of 140 samples including 120 RTE meat products (beef luncheon, chicken luncheon and beef frankfurter, 40 samples, each) and 20 hand swabs of their handlers were collected from randomly selected supermarkets at Al-Salam city, Cairo, Egypt. These samples were examined for the presence of Shiga toxins producing *E. coli* (*Stx*1 and *Stx*2) and enterotoxigenic *Staphylococcus aureus*. In addition, nasal swabs of RTE meat handlers were examined for enterotoxigenic *S. aureus*. Moreover, Stool of 50 diarrheic persons having the habit of eating RTE meat of the same categories examined were collected from private laboratories then examined for STEC. *E. coli* was detected in beef luncheon (20%), chicken luncheon (10%), stool samples (20%) and hand swabs (15%). Serological identification of *E. coli* isolates revealed the presence of *E. coli* O55:K59, O26:K60, O111:K58, O124:K72 and O128:K67 in beef luncheon, O55:K59 and O111:K58 in chicken luncheon, O55:K59, O124:K72 and O128:K67 in stool samples and O111:K58 and O124:K72 in hand swab. *Stx*1 was detected in O26:K60 isolate, *Stx*2 was detected in *E. coli* O128:K67 isolates, while *Stx*1 and *Stx*2 were detected in O111:K58 isolates. Moreover, *S. aureus* was detected in beef and chicken luncheon (10%, each), hand swabs (20%) and nasal swabs (30%). Further analysis of *S. aureus* isolates for toxigenic capabilities using ELISA technique revealed that the number of the enterotoxigenic strains were 3 (SEA, SED, SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon, 3 (SEA, SEC and SED) in hand swabs and 3 (SEA, SEC and SEA+SSED) in nasal swabs.

**Key words:** Ready-To-Eat Meat • Shiga Toxins-Producing *E. coli* • Enterotoxigenic *S. aureus* • Zoonoses

**INTRODUCTION**

Ready-to-eat (RTE) food products constitute a source of readily available and nutritious meals for wide scale consumers. Conversely, they are also considered as an ideal culture media for growth of many microorganisms and a major source of food borne pathogens [1].

Shiga toxin-producing *Escherichia coli* (STEC) represents one of the at least six different categories of diarrhoeagenic *E. coli* recognized [2]. Certain strains of STEC are frequently identified as causative agents of life-threatening diseases in humans, such as hemorrhagic colitis (HC) and hemolytic uraemic syndrome (HUS) [3]. A large number of STEC serogroups as non-O157:H7 (O26: H11, O111: H2, O103: H2, O128 and O145:H28) have been associated with food borne illnesses and their importance is increasing worldwide [4]. Transmission of infection to human occurs mainly through contaminated food of animal origin as well as cross contamination due to inappropriate food handling [5]. The majority of human diseases are associated with strains of STEC that produce either Shiga toxin 1 (*Stx*1) and/or Shiga toxin 2 (*Stx*2) which are encoded on lysogenic bacteriophages. A facet of STEC that makes them particularly worrisome food...
borne pathogens is their ability to survive the acidic milieu of various foods and human stomach [6].

Staphylococcal food poisoning is one of the most prevalent causes of gastroenteritis that is caused by the ingestion of food containing pre-formed toxins [7]. Despite the decrease in number of outbreaks reported annually in the last few decades, staphylococcal food poisoning is still reported as the third most prevalent cause of food borne illnesses worldwide [8]. Although staphylococci are commonly found on the skin of a wide variety of mammals and birds and on environmental surfaces, humans are thought to be the primary source of strains associated with food matrix staphylococcal intoxication [9]. Studies have shown that one of the most common types of food intoxication is caused by certain staphylococcal strains, mainly Staphylococcus aureus (S. aureus). Of the many extracellular toxins, staphylococcal enterotoxins (SE’s) pose the greatest risk to consumer's health [10]. These enterotoxins are highly resistant to heat, therefore, measures to prevent the growth of S. aureus are critical because normal cooking’s temperature will not destroy the toxins. Various typing methods have been developed to characterize S. aureus isolates. PCR and ELISA have been used as simple techniques for detecting enterotoxigenic strains [11].

The aim of this study was to investigate the prevalence of non-O157 STEC and enterotoxin producing S. aureus in some ready-to-eat meat products and human beings. A second aim was to characterize the virulence potential of any isolated STEC and enterotoxins producing S. aureus.

**MATERIALS AND METHODS**

**Samples Collection:** From June to August 2012, a total of 140 samples including 120 RTE meat products (beef luncheon, chicken luncheon and beef frankfurter; 40 samples, each) and 20 hand swabs of their handlers were collected from randomly selected supermarkets at Al-Salam city, Cairo, Egypt. RTE meat products and hand swabs were examined for the presence of STEC and enterotoxigenic S. aureus. In addition, nasal swabs of RTE meat handlers were collected and examined for enterotoxigenic S. aureus. Moreover, Stool samples of 50 diarrheic persons having the habit of eating RTE meat of the same categories examined were collected from private laboratories then examined for STEC.

**Isolation of E. Coli:** Briefly, 1 part of RTE meat products or stool samples and hand swabs were added to 9 parts of buffered peptone water (BPW), homogenized well, then incubated at 37°C for 24 h. A loopful of the pre-enriched homogenate was streaked onto MacConkey agar (Oxoid, CM7) and incubated for 24 h at 37 °C [12]. After incubation, suspected colonies were selected and identified biochemically by API 20 E (BioMerieux SA, France).

**Serotyping of E. coli Isolates:** E. coli isolates were serogrouped according to Kok et al. [13] using rapid diagnostic E. coli antisera sets (DIFCO Laboratories, Detroit Michigan 48232-7058, USA) at Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt. The serologically identified E. coli isolates were analyzed for the presence of Stx1 and Stx2 genes. Genomic DNA was extracted from each E. coli isolate using Bacterial DNA extraction kit (Spin-column) (BioTeke Corporation, Catalogue # DP2001) according to the manufacturer's instructions. The primers used for the detection of toxin genes have been published by Paton et al. [14] and Gannon et al. [15] as shown in Table 1.

**Pcr Assay for Detection of Stx1:** The reaction was performed in a volume of 20 µl containing 10 µl of readymade 2X power Taq PCR master mix (BioTeke Corporation, China), 20 µM Stx1 primers and 2 µl of the purified DNA. The reaction conditions consisted of one cycle of 95 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 5 min [15]. The reaction was carried out in Primus (MWG-Biotech) thermal cycler.

**Pcr Assay for Detection of Stx2:** A reaction volume of 20 µl containing 10 µl of readymade 2X power Taq PCR master mix (BioTeke Corporation, China) and 20 µM of primers (Alpha DNA, Canada) was used. The reaction conditions were: 25 cycles beginning with a 30-sec denaturation at 94 °C, primer annealing at 50 °C for 45 sec, followed by extension for 1 min 30 sec at 70 °C, a final extension for 10 min at 70 °C was also performed [16]. Amplification products were resolved in 1.2 % agarose gel, containing 5 µg ethidium bromides along with 100 bp molecular weight ladder (BioTeke Corporation, China). Agarose gels were run in 1X TBE, 5 µM ethidium bromide for at least 45 min at 100 volts, then visualized under Ultraviolet Transilluminator (Spectroline) and photographed.

**Isolation of S. aureus:** RTE meat samples, hand and nasal swabs were pre-enriched in BPW. A loopful of
pre-enriched samples in BPW were inoculated onto Baird parker agar medium (Oxoid, CM0275) [17] and incubated at 37 °C for 24 h. The suspected colonies were subjected to biochemical identification using catalase test, coagulase test (Test tube method) and oxidase test [18].

**Detection of Staphylococcal Enterotoxins:** For detection of enterotoxigenic capabilities, each isolate of *S. aureus* was incubated in Brain Heart infusion broth (DIFCO, USA) for 12 h at 37°C. The broth culture was then centrifuged at 3500 r.p.m. for 10 minutes. Sterile filtration of the supernatant was applied. The enzyme immunoassay (ELISA) was carried out in an ELISA plate reader (ELX800, BioTek Instruments, Bad Friedrichshall, Germany) where the absorbance was measured at 450 nm using the RIDASCREEN set A, B, C, D, E (Art No. R4101, R-Biopharm AG, Darmstadt, Germany) [19].

### RESULTS

*E. coli* was detected in 12 RTE meat products out of 120 examined (10%). The isolated *E. coli* were 8/40 beef luncheon (20%) and 4/40 chicken luncheon (10%). Beef frankfurter samples were free from *E. coli*. *E. coli* isolated from beef luncheon were serologically identified as O55:K59, O26:K60, O111:K58, O128:K67 (1 isolate, each) and O124:K72 (4 isolates). However, those isolated from chicken luncheon were identified as O55:K59 (3 isolates) and O111:K58 (1 isolate). PCR analysis of *E. coli* serotypes revealed the presence of Stx1 gene in O26:K60 isolate, Stx2 gene in O128:K67 isolates and both toxins in O111:K58 isolates (Table 2 and Figure 1).

Also *E. coli* was detected in 10/50 stool samples (20%) and 3/20 hand swabs of RTE meat handlers examined (15%). *E. coli* isolates from stool were serologically identified as O55:K59 (4 strains, 8%), O124:K72 (5 strains, 10%) and O128:K67 (1 strain, 2%). However, those from hand swab were O111:K58 (1 strain) and O124:K72 (2 strains). Stx2 was detected in O128:K67 isolate from diarrheic stool, while Stx1 and Stx2 were detected in O111:K58 from hand swabs (Table 3 and Figure 1).

*S. aureus* was detected in beef and chicken luncheon (10%, each) Table (4). Further analysis of *S. aureus* isolates from RTE meat products samples for toxigenic capabilities using ELISA technique revealed the presence of 3/4 enterotoxigenic strains (SEA,SED,SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon (Table 4).

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### Table 1: Oligonucleotide primers of Stx1 and Stx2 genes of E. coli:

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Oligonucleotide sequence (5’-3’)</th>
<th>Predicted size of amplified products (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stx1</td>
<td>TTC GCT CTG CAA TAG GTA</td>
<td>555</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>TTC CCC AGT TCA ATG TAA GAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx2</td>
<td>CCA TGA CAC CGG ACA GCA GTT</td>
<td>779</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>CCT GTC AAC TGA GCA TTG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Table 2: Prevalence of Stx1 and Stx2 producing E. coli in RTE meat products

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of positive samples (%)</th>
<th>The identified serotypes</th>
<th>No. (%)*</th>
<th>E. coli biotype</th>
<th>Stx1</th>
<th>Stx2</th>
<th>Shiga toxins production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef luncheon (n=40)</td>
<td>8 (20)</td>
<td>O55:K59</td>
<td>1(2.5)</td>
<td>EPEC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O26:K60</td>
<td>1(2.5)</td>
<td>EHEC</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O111:K58</td>
<td>1(2.5)</td>
<td>EHEC</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O124:K72</td>
<td>4(10)</td>
<td>EIEC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O128:K67</td>
<td>1(2.5)</td>
<td>ETEC</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chicken luncheon (n=40)</td>
<td>4 (10)</td>
<td>O55:K59</td>
<td>3(7.5)</td>
<td>EPEC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O111:K58</td>
<td>1(2.5)</td>
<td>EHEC</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Beef frankfurter (n=40)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*The percentage of the identified serotypes was calculated from the total examined samples

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### Table 3: Prevalence of Stx1 and Stx2 producing E. coli in hand swabs of RTE meat handlers and stool samples of consumers

<table>
<thead>
<tr>
<th>Examined Samples</th>
<th>No. of positive samples (%)</th>
<th>The identified serotypes</th>
<th>No.</th>
<th>Percent to total examined samples</th>
<th>E. coli biotype</th>
<th>Stx1</th>
<th>Stx2</th>
<th>Shiga toxin production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool samples (n=50)</td>
<td>10 (20)</td>
<td>O55:K59</td>
<td>4</td>
<td>8</td>
<td>EPEC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O124:K72</td>
<td>5</td>
<td>10</td>
<td>EIEC</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Occurrence of enterotoxigenic S. aureus in RTE meat products and hand and nasal swabs of their handlers.

<table>
<thead>
<tr>
<th>Source Examined samples (%)</th>
<th>S. aureus positive samples (%)</th>
<th>Enterotoxins type (No. of isolates producing the respective toxin)</th>
<th>Total (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>40</td>
<td>4 (10)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Beef luncheon</td>
<td>40</td>
<td>4 (10)</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Chicken Luncheon</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Beef frankfurter</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>8 (6.7)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Hand swabs</td>
<td>20</td>
<td>4 (20)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Nasal swabs</td>
<td>20</td>
<td>6 (30)</td>
<td>3 (50)</td>
</tr>
</tbody>
</table>

* The percentage of the enterotoxigenic S. aureus isolates were calculated to the total positive samples.

**Fig. 1: PCR results of E. coli serotype positive for Stx1 and Stx2.** L: 100 bp Ladder; Lane 1: Negative control; Lanes 2-6, E. coli samples positive for Shiga toxin 2 gene (Stx2) at 779 bp; Lane 2: O111:K58 from hand swab; Lane 3: O111:K58 from beef luncheon; Lane 4: O111:K58 from chicken luncheon; Lane 5: O128:K67 from beef luncheon; Lane 6: O128:K67 from stool samples; Lanes 7-10, E. coli samples positive for Shiga toxin 1 gene (Stx1) at 555 bp; Lane 7: O111:K58 from hand swab; Lane 8: O111:K58 from beef luncheon; Lane 9: O111:K58 from chicken luncheon; Lane 10: O26:K60 from beef luncheon.

**DISCUSSION**

Several reports have documented the isolation and serotyping of E. coli in beef meat products [20-22] and chicken meat products [23, 24]. However, limited information is available on the genotypic characterizations of Stx1 and Stx2 producing E. coli. Such studies are important because STEC cause life threatening infections when transmitted to man through contaminated food of animal origin. The results of the present study demonstrated that only 8/40 beef luncheon (20%) were positive for E. coli (Table 2). Similar prevalence of E. coli in beef products was recorded in Giza, Egypt by AL-Mutairi [22] who detected E. coli in 15/75 beef meat products (20%). His samples were processed kofta, sausage and shawerma that were sold by street vendors who were not observing the basic rules of personal hygiene. Higher prevalence rate (30.8%) of E. coli in beef meat products were recorded in Sharkia, Egypt by Mohamed et al. [25]. The samples they examined were frozen and baladi sausage which were not subjected to heat treatment during manufacturing. On the contrary, lower prevalence (2%) was recorded by Hassanien [26] in Qalyobia, Egypt.

In Egypt, there are few reports describing isolation of STEC other than E. coli O157:H7 from beef meat products, even though the transmission of non-O157 STEC to human has been associated with consumption of beef meat products [27]. In this study, non-O157 STEC (O26:K60, O111:K58 and O128:K67) were isolated from beef luncheon. Non-O157:H7 STEC were previously recorded in beef meat products [21, 22, 28]. Hussein and Bollinger [20] found non-O157 STEC to be more prevalent in beef products than E. coli O157. They also stated that the prevalence rates of non-O157 STEC ranged from 1.7 to 58% in packing plants, 3 to 62.5% in supermarkets and an average of 3% in fast food restaurants.

Regarding the prevalence of E. coli in chicken luncheon, Table 2 reveals the isolation of E. coli from 10% of the examined samples (4/40). These isolates were identified as O55:K59 (3 isolates) and O111:K58 (1 isolate). Lower prevalence (2%) was recorded by Zahran [29] in Qalyobia, Egypt. On the contrary, higher prevalence (25%) was recorded by Sharaf and Sabra [30] in Al-Taif, Saudia Arabia. El-Nawawi et al. [24] isolated O44 from
chicken meat products in Egypt.

Absence of E. coli isolates in beef frankfurter (Table 2) may be attributed to purchasing of beef frankfurter samples in their original intact package; consequently they were not subjected to cross contamination by slicing machines or contaminated hands. These results agreed with Javadi et al. [31].

E. coli O55:K59 and O111:K58 isolated from beef and chicken luncheon were categorized as enteropathogenic Escherichia coli (EPEC) and enterohaemorrhagic Escherichia coli- Shiga toxigenic Escherichia coli (EHEC-STEC), respectively. Alonso et al. [23] in Argentina reported a predominance of EPEC contamination in chicken meat and chicken meat products. The occurrence of EPEC and STEC in chicken meat may be related to the evisceration process, mainly due to the rupture of the intestine, or possible cross contamination during food processing and handling. The presence of STEC O55:K59 and O111:K58 in beef and chicken luncheon in this study may be attributed to that the grinding machine used to prepare chicken luncheon is shared with bovine meat or due to addition of bovine fat to chicken meat for thorough mixing of the latter.

Table 3 shows that E. coli was detected in 10/50 stool samples (20%). E. coli isolates from stool were serologically identified as O55:K59 (4 strains, 8%), O124:K72 (5 strains, 10%) and O128:K67 (1 strains, 2%). In comparison to this study, higher prevalence rate (51.5%) of E. coli was recorded in Cairo by Behiry et al. [32]. However, lower prevalence (6%) was previously recorded by Bodhidatta et al. [33]. Generally, the variation in the prevalence rate of E. coli from one study to another may be accounted for differences in number and health status of human cases examined, localities and hygienic measures.

Also E. coli was detected in 15% of hand swabs of RTE meat handlers and were identified as O111:K58 (1 strain) and O124:K72 (2 strains) (Table 3). Samaha et al. [34] and Mohamed et al. [25] isolated E. coli from hand swabs of food handlers in Egypt. Their respective results were 7.5 and 32%. The difference in the prevalence rates may be related to the kind of food they handled.

E. coli O128:K67 isolated from diarrheic stools and beef luncheon was categorized as ETEC and was found to contain Stx2 that is more related to HUS than Stx1 producing strains [35]. Meanwhile, E. coli O124:K72 isolated from stool, hand swabs and beef luncheon was categorized as enteroinvasive E. coli (EIEC). Humans are a major reservoir of EIEC and the serotypes most frequently associated with illness include O143, O144, O152, O164 and O167. Among these serogroups, O124 is commonly encountered [36]. ETEC were recorded in stool samples in previous studies [37].

PCR analysis of E. coli isolates for the presence of Stx1 and Stx2 genes clarified the presence of Stx1 gene in O26:K60 isolate, Stx2 gene in O128:K67 isolates and both toxins in O111:K58 isolates (Figure 1). Isolation of the Stx1 and Stx2 producing E. coli O111:K58 from beef and chicken luncheon and hand swabs of food handlers in the current study suggests cross contamination from handlers to food and vice versa. Moreover, isolation of O124:K72 from beef luncheon, hand swabs of RTE meat handlers and stool of diarrheic persons trigger the transmission cycle from infected handlers to food products and finally to consumers.

Enterotoxigenic S. aureus is one of the causative agents of food borne intoxication. For this reason determination of its prevalence in foods is important with respect to assessing public health risk. Bryan [38] observed that of 175 staphylococcal outbreaks, 29% were traced to ham and 8% to chicken dishes. In this study, S. aureus was detected in beef and chicken luncheon (10%, each) Table (4). In previous study, coagulase positive S. aureus was detected in 7110 (6.4%) hamburger patties in Turkey [39].

Further analysis of S. aureus isolates from RTE meat products samples for toxigenic capabilities using ELISA technique revealed the presence of 3/4 enterotoxigenic strains (SEA, SED, SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon (Table 4). In spite of the low prevalence of S. aureus in RTE meat products the proportion of enterotoxigenic strains is considered high. The obtained results agreed with Bania et al. [40] who reported that 15-80% of S. aureus strains isolated from various sources are enterotoxigenic. It is very likely that contamination of RTE meat products occurred during handling and mixing of ingredients. This stresses the need to instruct handlers on how to implement good manufacturing practices.

Hands and mucous membranes of the nasopharynx are considered the predominant colonization sites of staphylococci. The source of S. aureus intoxication is any kind of food which has come into contact with food handlers’ hands contaminated with S. aureus and subsequently not properly stored [41]. S. aureus was detected in 4 hand swabs (20%) and in 6 nasal swabs (30%) of meat handlers. The enterotoxin typing of S. aureus isolates revealed the presence of 3/4 enterotoxigenic strains (75%) in hand swabs (SEA, SEC.
and SED) and 3/6 enterotoxigenic strains (50%) in nasal swabs examined (SEA, SEC and SEA+SED). Enterotoxigenic *S. aureus* was previously detected in hand swabs of food handlers; SEA [42], SEB [43], SEC [44], SED [45]. A recent study in Botswana reported that 57.5% of the food handlers harbored *S. aureus* and 21% of them possessed toxigenic strains [46]. Isolation of enterotoxigenic *S. aureus* from nasal swabs agreed with Udo *et al.* [43] and Soriano *et al.* [45]. Isolation of enterotoxigenic *S. aureus* from nasal and hand swabs of RTE meat handlers, chicken and beef luncheon substantiate the role played by food handlers in dissemination of such bacteria through foods they handle.

**CONCLUSION**

It could be concluded that *E. coli* and *S. aureus* harboring toxin genes are prevalent in Beef and chicken luncheon and humans in Egypt. The obtained data illustrate the need to keep a careful watch over which pathogens are causing human disease, understanding STEC and enterotoxigenic *S. aureus* epidemiology and a control strategy plan. Furthermore, it is convenient for the authors to suggest beef frankfurter as a safer meat product depending on the obtained results of this study and similar ones that illustrate absence of both non-O157 STEC and enterotoxigenic *S. aureus*.

**REFERENCES**


