Clostridium difficile is an important gastrointestinal pathogen associated with, but not limited to, diarrheal disease of humans, horses, and pigs (1,2). It is of growing concern in human healthcare due to the increase in frequency and severity of C. difficile infection (CDI) and apparent increase in community-associated (CA) C. difficile can be isolated from varying percentages of food and food producing animals, and often these isolates are indistinguishable from those implicated in human disease. A particular concern is the isolation of ribotype 027, and another highly virulent isolate, 078, from meat (4). The presence of important C. difficile strains in food sources has raised concern about the potential for foodborne transmission. In addition to meat, C. difficile has also been reported in salads (5) yet there has been limited investigation of other possible food sources. The objective of this study was to determine if C. difficile could be isolated from a variety of retail vegetables, seafood and fish and to characterize these isolates.

Materials and Methods

Sample collection and processing. Vegetable samples were collected between May and August, 2009 and seafood and fish samples were collected between May and August, 2010. A convenience sample of 111 vegetable and 86 seafood samples were purchased from 11 different grocery stores around Guelph, Ontario, Canada. Additionally, 33 fish samples were purchased from outlets using the systematic sampling method used for the retail component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (6). Vegetables were incubated in 50 ml of C. difficile growth medium (11) anaerobically at 37°C for 7 days. Approximately 15 g of vegetable or fish tissue was inoculated into 50 ml of the same medium and incubated under the same conditions. Two ml of each culture were then mixed with an equal volume of ethanol, vortexed briefly and incubated at room temperature for 1 hour. Cultures were then centrifuged and the pellet was plated onto blood agar plates and incubated anaerobically at 37°C for another 48 hours. C. difficile probable colonies were identified by colony morphology and odor, and were subcultured onto blood agar plates and incubated anaerobically at 37°C. Identification was confirmed by a positive L-proline aminopeptidase test (Predski, Remel, Lenexa, KS, USA) and amplification of the triose phosphate isomerase gene (7).

Isolate characterization. Detection of tcdA, tcdB, cdtB, and ribotyping were performed as previously described (7,8,9). Toxotyping was performed as described by Pirs et al. (10). MICs for clindamycin and metronidazole, vancomycin, and levofloxacin was determined using E-tests (AB Biodisk, Solna, Sweden). Breakpoints used were in accordance with Martin et al. (2008) (11).

Discussion

• The most commonly found ribotype in both seafood and vegetable samples, 078, is thought to be hypervirulent and is associated with severe CDI, suggesting a possible human health concern. This ribotype has been reported to be the predominant type in food animals such as cattle and pigs (3, 12, 13).

• Although, C. difficile prevalence between root and non-root vegetables was not significant, the P-value suggests that further study of the relative risk from different types of vegetables should be considered.

• It is interesting to note that 4/5 isolates in the vegetable study were imported from outside of North America. It is unclear whether this indicates a greater risk from imported produce or if it is related to the types of products that are imported.

• This study indicates that contamination of retail vegetables, seafood, and fish can occur. Currently, the public health relevance is unclear since food hasn't been proven to be a source of infection.

Results

• C. difficile was isolated from 5/111 (4.5%) retail vegetable samples and 5/119 (4.8%) seafood and fish samples (Table 1).

• In vegetables, 3/5 isolates and in fish 4/5 isolates were ribotype 078/NAP 7/toxotype V (Figure 1).

• All toxigenic strains have been previously found in humans with CDI in Ontario, Canada.

• A non-significant difference was found when root and non-root vegetables with respect to C. difficile contamination were compared (P=0.068).

• The prevalence of contamination was higher in imported vegetables compared to those from Canada (P=0.016).

• MICs for 4 antimicrobials are presented in Table 2. All strains isolated from retail vegetable samples. B. Ribotyping profiles of the 5 C. difficile strains isolated from retail seafood and fish. M: 100 bp marker, 78: internationally recognized ribotype 078/NAP 7/toxotype V strain; V: internal laboratory ribotype designation, OVC0: ribotype profile not similar to any known ribotype.

• Isolation is of concern, however, that the majority of isolates found in these food sources are indistinguishable from ribotype 078, a hypervirulent strain associated with severe disease in humans and only 1 isolate was found to be non-toxigenic.

• The potential sources of contamination of food are various and can include source contamination from agricultural run-off and/or cross-contamination during processing and sale.

• This study combined with other studies demonstrating C. difficile contamination of various meats indicates contamination of food is common. Further study of the role of food in human C. difficile infection is required.

Acknowledgements

We also thank the CIPARS-Retail program for providing some seafood and fish samples. This study was supported, in part, by the Public Health Agency of Canada.

References


Table 1. Characterization of the 5 vegetable and 5 seafood isolates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Country</th>
<th>Ribotype</th>
<th>Toxin Genes</th>
<th>Toxinotype</th>
<th>NAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginger (078)</td>
<td>China</td>
<td>078</td>
<td>tcdA, tcdB, cdtB</td>
<td>V, 7</td>
<td></td>
</tr>
<tr>
<td>Carrot (078)</td>
<td>USA</td>
<td>078</td>
<td>tcdA, tcdB, cdtB</td>
<td>V, 7</td>
<td></td>
</tr>
<tr>
<td>Ginger (078)</td>
<td>USA</td>
<td>078</td>
<td>tcdA, tcdB, cdtB</td>
<td>V, 7</td>
<td></td>
</tr>
<tr>
<td>Eddoes (China)</td>
<td>China</td>
<td>078</td>
<td>tcdA, tcdB, cdtB</td>
<td>V, 7</td>
<td></td>
</tr>
<tr>
<td>Shrimp (078)</td>
<td>USA</td>
<td>078</td>
<td>tcdA, tcdB, cdtB</td>
<td>V, 7</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. MICs of vegetables and seafood isolates.

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Levo</th>
<th>Clinda</th>
<th>Metro</th>
<th>Vanc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginger (078)</td>
<td>3.0 (S)</td>
<td>0.125 (S)</td>
<td>1.5 (S)</td>
<td>2.0 (I)</td>
</tr>
<tr>
<td>Carrot (078)</td>
<td>3.0 (S)</td>
<td>0.125 (S)</td>
<td>1.5 (S)</td>
<td>2.0 (I)</td>
</tr>
<tr>
<td>Ginger (V)</td>
<td>6.0 (S)</td>
<td>0.125 (S)</td>
<td>1.5 (S)</td>
<td>2.0 (I)</td>
</tr>
<tr>
<td>Shrimp (078)</td>
<td>0.094 (S)</td>
<td>0.125 (S)</td>
<td>1.5 (S)</td>
<td>2.0 (I)</td>
</tr>
</tbody>
</table>

Key: NT: not tested, NAP: North American Pulsotype

Figure 1. A. Ribotyping profiles of the 5 C. difficile strains isolated from retail vegetable samples. B. Ribotyping profiles of the 5 C. difficile strains isolated from retail seafood and fish. M: 100 bp marker, 078: internationally recognized ribotype 078/NAP 7/toxotype V strain; V: internal laboratory ribotype designation, OVC0: ribotype profile not similar to any known ribotype.

Figure 1.