Clostridium difficile in vegetables and seafood

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Introduction

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 disease (3). *C. difficile* can be isolated from varying percentages of food and food producing animals, and often these isolates are indistinguishable from strains implicated in human disease. Of 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 seafood or fish were 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 odour, and were subcultured onto blood agar plates and incubated anaerobically at 37°C. Identification was confirmed by a positive L-proline aminopeptidase test (Prodisk, 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). Toxinotyping was performed as described by Rupnik et al. (10). MICs for clindamycin, metronidazole, vancomycin, and levofloxacin was determined using E-tests (AB Biodisk, Solna, Sweden). Breakpoints used were in accordance with Martin et al. 2008 (11).

Table 2. MICs of vegetables and seafood isolates.

	Levo	Clinda	Metro	Vanco
Ginger (078)	3.0 (I)	6.0 (I)	0.19 (S)	1.0 (S)
Carrot (078)	3.0 (I)	8.0 (R)	0.125 (S)	0.75 (S)
Ginger (V)	6.0 (I)	8.0 (R)	1.5 (S)	1.5 (S)
Eddoes (V)	6 .0 (I)	8.0 (R)	1.5 (S)	1.5 (S)
Ginger (078)	0.75 (S)	8.0 (R)	0.38 (S)	0.19 (S)
Scallop (078)	NT	0.125 (S)	0.25 (S)	0.75 (S)
Perch (078)	NT	0.94 (S)	0.38 (S)	1.5 (S)
Shrimp (unknown)	NT	0.064 (S)	1.0 (S)	1.0 (S)
Salmon (078)	NT	0.5 (S)	0.19 (S)	1.5 (S)
Cooked shrimp (078)	NT	1 (S)	0.094 (S)	0.5 (S)

Key: I-intermediate resistance, R-resistant, S-susceptible, ribotype in parentheses, levo – levofloxacin, clinda-clindamycin, metro-metronidazole, vanco-vancomycin, NT: not tested

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/toxinotype 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 isolates were fully susceptible to metronidazole and vancomycin. Resistance to clindamycin was observed in 3 vegetable isolates but no seafood isolates.

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

Sample	Country	Ribotype	Toxin Genes	Toxinotype	NAP
Vegetables					
Ginger	China	078	*tcdA, tcdB, cdtB*	V	7
Carrot	USA	078	*tcdA, tcdB, cdtB*	V	7
Ginger	China	RV	*tcdA, tcdB*	0	4
Eddoes	China	RV	*tcdA, tcdB*	0	4
Ginger	China	078	*tcdA, tcdB, cdtB*	V	7
Seafood/Fish					
Frozen scallop	Unknown	078	*tcdA, tcdB, cdtB*	V	NT
Perch	Canada	078	*tcdA, tcdB, cdtB*	V	7
Shrimp	Vietnam	OVCO	-	NT	NT
Salmon	Canada	078	*tcdA, tcdB, cdtB*	V	7
Cooked shrimp	Canada	078	*tcdA, tcdB, cdtB*	V	7

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

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.

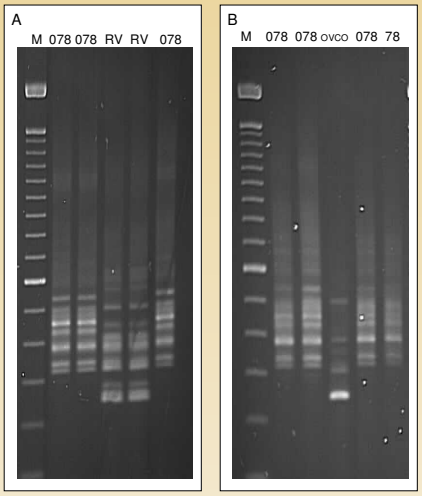

Figure 1. A. Ribotyping profiles of the *5 C. difficile* strains isolated from retail vegetable samples. B. Ribotyping profiles of the 5 *C. difficile* strains isolated from retail seafood and fish. M: 100 bp marker, 078: internationally recognized ribotype 078/NAP 7/toxinotype V strain, V: internal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

- It 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.

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References

1) Arroyo, L. et al. 2005. J. Med. Microbiol. 54:163-166. 2) Keel, K. et al. 2007. J. Clin. Microbiol. 45:1963- 1964. 3) McDonald, L., *et al.* 2005. NEJM. 353:2433-2441. 4) Rupnik, M. 2007. Clin. Microbiol. Infect. 13:457-459. 5) Bakri, M. et al. 2009. Emerg. Infect. Dis. 15:817-818. 6) Government of Canada. 2007. http://www.phac-aspc.gc.ca/cipars-picra/surv_retail_e.html . 7) Bidet, P. et al. 1999. FEMS Microbiol. Lett.175:261-266. 8) Stubbs, S *et al.* 2000. FEMS Microbiol. Lett. 186:307-212. 9) Lemee, L *et al.*, 2004 J. Clin. Microbiol. 42:5710-5714. 10) Rupnik, M *et al.* 1998. J. Clin. Microbiol. 36:2240-2247. 11) Martin, H. et al. 2008. J. Clin. Microbiol. 46:2999-3004.12) Pirs, T. et al. 2007. J. Med. Microbiol. 57:790-792. 13) Rupnik, M., et al. 2008, J .Clin. Microbiol. 46:1963-1964.