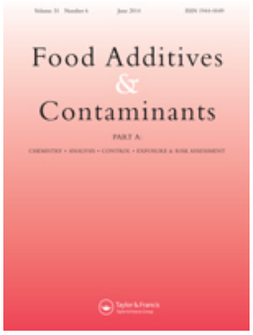

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Survey of *Clostridium difficile* in retail seafood in College Station, Texas

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The incidence and severity of disease associated with toxigenic *Clostridium difficile* have increased in hospitals in North America with the emergence of newer, more virulent strains. Toxigenic *C. difficile* has been isolated from food animals and retail meat with potential implications of transfer to humans. The objective of the present study was to investigate the prevalence of *C. difficile* in retail seafood from grocery stores in College Station, Texas. *C. difficile* was found in 4.5% (3/67) of shellfish and finfish samples. The positive samples included one each from fresh mussel, frozen salmon and frozen shrimp. The mussel and salmon isolates were characterized as toxinotype V and pulsed-field gel electrophoresis (PFGE) type-NAP7. The shrimp isolate was identified as toxinotype XII, but had an unknown PFGE type. Susceptibilities to 11 antimicrobial agents were identical for the mussel and salmon isolates and were sensitive to eight of 11 antimicrobials (including ampicillin) and intermediate to clindamycin. However, the shrimp isolate was resistant to clindamycin and ampicillin. This study demonstrates that seafood, like other food commodities, can be contaminated by *C. difficile*.

Keywords: *Clostridium difficile*; finfish; retail grocers; shellfish; toxinotype

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Introduction

Since 2003, the incidence and severity of disease associated with toxigenic *Clostridium difficile* have increased in hospitals of North America. These increases suggest the emergence of a new strain of toxigenic *C. difficile* – characterized as restriction endonuclease analysis type BI, North American pulsed-field gel electrophoresis (PFGE) pattern 1 (BI/NAP1), toxinotype III – that exhibits increased levels of resistance, virulence and toxin production (McDonald et al. 2005). Various strains of *C. difficile*, including NAP1, toxinotype III, can be isolated from food animals and meat (Weese 2010); however, the predominant strains from food animals are NAP7 and NAP8, toxinotype V (Jhung et al. 2008). Toxigenic strains of the bacterium have been isolated globally from food-producing animals, meat and poultry (Weese 2010; Harvey, Norman, Andrews, Hume, et al. 2011; Harvey, Norman, Andrews, Norby, et al. 2011), from seafood in Canada (Metcalf et al. 2011), and from marine molluscs in Italy (Pasquale et al. 2012). Speculation has it that *C. difficile* may be a food-associated organism and consumption of contaminated products could be responsible for increased community-associated *C. difficile* infection (Jhung et al. 2008). The objective of the present study was to determine the prevalence of toxigenic *C. difficile* in commercially sold shellfish and finfish from grocery stores in College Station, Texas.

Materials and methods

On three different occasions in March, April and December 2012, the authors, using a convenience sample plan, purchased a total of 67 seafood samples from three separate grocery stores in College Station, TX. Stores 1 and 2 were from a regional grocery chain, whereas store 3 was from a national chain. Samples consisted of live clams, mussels, crawfish and fresh shucked oysters (canned) (sample size = minimum of 20 each); fresh rainbow trout (whole and fillets), turbot, tilapia, steelhead trout, catfish, red snapper and halibut (sample = 500 g each); and frozen blue claw crab, sockeye salmon, Atlantic and Alaskan cod, pink salmon, tilapia, whiting, shrimp (both raw and cooked), halibut, grouper, ocean perch, mahi-mahi, orange roughy, snow crab legs and claws, crawfish tail meat (cooked), calamari, mussels (cooked), striped Pangasius (swai), bay scallops, whole brown clams (cooked), and sea scallops (sample = 500 g each).

Cultivation techniques for *C. difficile* were a slight modification of that previously described (Metcalf et al. 2011). Briefly, 70 mg seafood were inoculated into 100 ml of pre-reduced *C. difficile* enrichment broth medium comprised of 40 g l^{-1} proteose peptone, 5.0 g l^{-1} disodium hydrogen phosphate, 1.0 g l^{-1} potassium dihydrogen phosphate, 0.1 g l^{-1} magnesium sulfate, 2.0 g l^{-1} sodium chloride, 6.0 g l^{-1} fructose, 0.1% sodium taurocholate and 0.5 g l^{-1} cysteine hydrochloride supplemented with moxalactam (32 mg l^{-1}) and norfloxacin (12 mg l^{-1})

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(Sigma, St. Louis, MO, USA), which was incubated for 7 days in an anaerobic chamber (5% CO_2, 5% H_2, 90% N_2) at 37°C. A total of 2 ml of each inoculant were mixed with an equal volume of ethanol, vortexed, incubated at RT for 1 h and centrifuged at 3500*g* for 10 min. After centrifugation, all but 200 µl of the supernatant fluid was discarded. The pellet was resuspended in the remaining supernatant and pipetted onto *C. difficile* culture agar (Oxoid, Basingstoke, Hampshire, UK), supplemented with moxalactam, norfloxacin, (CDMN) and 5% laked horse blood (Lampire Biological Laboratories, Pipersville, PA, USA) and incubated anaerobically at 37° C for 72 h. Colonies that demonstrated characteristic morphology and odour for *C. difficile* were streaked directly onto CDMN and incubated in an anaerobic chamber overnight at 37°C.

Presumptive diagnosis consisted of the presence of a colony morphologically similar to *C. difficile*, L-proline aminopeptidase activity (Pro Disc, Key Scientific Products, Stamford, TX, USA) and biochemical characterization (API Rapid ID 32A, bioMerieux, Durham, NC, USA). PCR was used for detection of toxin A (*tcdA*) and B (*tcdB*) genes, *tcdC* gene deletion, toxinotype, and the *cdtB* binary toxin gene and have been described (Killgore et al. 2008; Harvey, Norman, Andrews, Hume, et al. 2011; Harvey, Norman, Andrews, Norby, et al. 2011). Each *C. difficile* isolate was tested for susceptibility to 11 antimicrobial agents by use of a commercially available test (E-test, bioMerieux, Durham, NC, USA) according to the manufacturer's recommendations and have been described (Norman et al. 2009; Harvey, Norman, Andrews, Norby, et al. 2011). PFGE was used to compare genotypes of *C. difficile* isolates. The PFGE procedures followed techniques of a modified 7-day protocol utilized by the Centers for Disease Control and Prevention (CDC; A. Thompson, personal communication, 2008) and have been described (Killgore et al. 2008; Harvey, Norman, Andrews, Norby, et al. 2011).

Results and discussion

Three of the 67 (4.5%) seafood samples from the grocers were positive for *C. difficile* (Figure 1). Two of the isolates (mussel and salmon) were characterized as toxinotype V and PFGE North America Pulsed-Field type (NAP) 7. The third isolate, from shrimp, was classified as toxinotype XII, but the PFGE type could not be identified. The positive samples consisted of a fresh mussel (farm-raised, Canada) from grocer 1 collected on 22 March, 2012; a frozen whole Pink salmon (wild-caught, Alaska) collected from grocer 2 collected on 16 April 2012; and a frozen shrimp with peeling and tail intact (wild-caught, Texas) collected from grocer 1 on 4 December 2012. None of the samples collected from grocer 3 (national chain) tested positive for *C. difficile*. Prevalence in the present study (4.5%) is almost identical to that of a Canadian study in

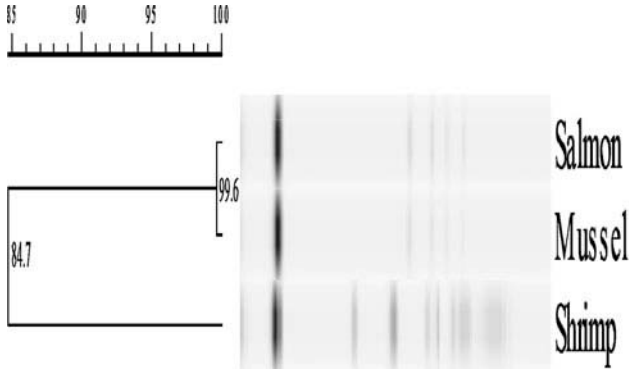

Figure 1. Pulsed field gel electrophoresis (PFGE) images and dendrogram of three *Clostridium difficile* isolates from retail seafood. The salmon (top lane) and mussel (middle lane) isolates are PFGE type-NAP7; the shrimp (bottom lane) is an unknown PFGE type.

which 4.8% (5/119) of retail seafood was positive for *C. difficile* (Metcalf et al. 2011). In that study, shrimp, scallops and salmon were contaminated, very similar to isolations from mussels, shrimp and salmon in the present study. Furthermore, the Canadian study reported that four of five of isolates were toxinotype V, with three of five PFGE NAP7, which agrees with the present study where two of three were NAP7.

Susceptibilities to 11 antimicrobial agents were identical for the mussel and salmon isolates and were sensitive to eight of 11 antimicrobials (including ampicillin) and intermediate to clindamycin (Table 1). However, the

Table 1. Antimicrobial susceptibility[a] of three *Clostridium difficile* isolates from retail seafood in College Station, Texas.[b]

	Sample		
Antimicrobial	Fresh mussel	Frozen salmon	Frozen shrimp
Cefoxitin	256 (R)	256 (R)	256 (R)
Tetracycline	0.094 (S)	0.047 (S)	1.5 (S)
Metronidazole	0.5 (S)	0.125 (S)	0.38 (S)
Clindamycin	3 (I)	4 (I) 256 (R)	–
Ampicillin	0.5 (S)	0.75 (S)	2 (R)
Chloramphenicol	3 (S)	4 (S)	6 (S)
Gatifloxacin	0.75 (S)	1 (S)	1 (S)
Piperacillin/tazobactam	2 (S)	2 (S)	12 (S)
Amoxicillin/clavulanic acid	0.19 (S)	0.25 (S)	1.5 (S)
Imipenem	32 (R)	32 (R)	32 (R)
Vancomycin	0.75 (S)	0.75 (S)	0.75 (S)

Notes: [a]Minimum inhibitory concentrations (µg ml^{-1}).
[b]Results are interpreted according to the Clinical and Laboratory Standards Institute: S, sensitive; I, intermediate; and R, resistant; gatifloxacin and vancomycin interpretation is based on values for trovafloxacin and Gram-positive aerobes, respectively (Clinical and Laboratory Standards Institute 2007).

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shrimp isolate was resistant to clindamycin and ampicillin. These results generally are similar to what has been reported for pork and pork sausage; however, differences were observed for ampicillin (83% I, 13% R), clindamycin (35% S, 57% I, 8.7% R), and tetracycline (74% S) (Harvey, Norman, Andrews, Norby, et al. 2011). On the other hand, the susceptibility of *C. difficile* from poultry meat to ampicillin was 100%, whereas clindamycin was observed at 28% I and 72% R (Harvey, Norman, Andrews, Hume, et al. 2011).

On the basis of the present study and that of Metcalf et al. (2011), overall prevalence of *C. difficile* in seafood appears to be low compared with that of 12.5–15% in poultry meat (Weese 2010; Harvey, Norman, Andrews, Hume, et al. 2011), 9.5% in pork and pork sausage (Harvey, Norman, Andrews, Norby, et al. 2011), and 42% in a variety of retail meats (Songer et al. 2009). However, prevalence for *C. difficile* in seafood of this study is minimal compared with the prevalence of *C. difficile* isolated from bivalve molluscs in Italy (Pasquale et al. 2012). In that study, 26/53 (49%) of samples were positive for *C. difficile*. Four of the 26 (15%) isolates were toxinotype V, two were ribotype 078, and 16/26 (62%) of isolates were grouped into 12 Cardiff standard PCR ribotypes (Anaerobe Reference Laboratory, Cardiff, UK). The high prevalence of *C. difficile* was attributed to raw sewage pollution from a river running into a bay where the sampling took place. Furthermore, the majority of isolate ribotypes closely resembled those from human cases of *C. difficile*-associated disease in that region of Italy.

Conclusions

The authors of the present study are at a loss to explain the significance of *C. difficile* presence in seafood. However, it should be recognized that *C. difficile* has been isolated from a myriad of unsuspected sources such as vegetables, salads and ready-to-eat meat products (Bakri et al. 2009; Songer et al. 2009; Metcalf et al. 2010). Because of the ubiquitous presence of *C. difficile*, these isolations may indicate that the organism is merely an environmental contaminant that enters the food chain through food processing facilities. It should also be noted that to date a food-borne *C. difficile* infection has not been reported.

The present study was limited by the bias in cultivation (media restrictions, colony numbers for selection, inoculation size, incubation time and temperature, etc.) Other limitations would include the small sample size and the limited geographical area for sample collection. Other studies attempting to isolate *C. difficile* from seafood would need to compare the similarities and differences in cultivation techniques before comparing prevalence. The authors are unsure of the clinical relevance of isolation of *C. difficile* from seafood as pertains to potential transfer of *C. difficile* from seafood to humans.

Acknowledgements

Proprietary or brand names are necessary to report factually on available data; however, the US Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, or exclusion of others that may be suitable. The authors declared no potential conflicts of interest with respect to the research, authorship, publication and/or financial support for this article.

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