Detection of _Clostridium difficile_ in retail ground meat products in Manitoba

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The aim of the present study was to determine whether _Clostridium difficile_ was present in uncooked retail ground beef and ground pork products sold in Winnipeg, Manitoba. Using an alcohol treatment protocol and inoculation of cultures on _Clostridium difficile_ Moxalactam Norfloxacain (CDMN), toxigenic _C. difficile_ was found in 6.3% of 48 meat samples. The _C. difficile_ isolates belonged to different pulsotypes, all of which had been previously isolated from the stool of Manitoba patients with _C. difficile_ disease. Because cooking of meat will not eradicate _C. difficile_ spores, this raises a concern regarding potential foodborne transmissibility of this organism.

Key Words: _C. difficile_; Food-borne disease; Zoonotic disease

_Clostridium difficile_ is an anaerobic spore-forming human pathogen associated with serious enteric diseases such as antibiotic-associated diarrhea and pseudomembranous colitis (1). Although _C. difficile_ infection (CDI) occurs mostly in health care facilities and is considered to be a nosocomial infection, the rate of community-acquired _C. difficile_ infection (CA-CDI) appears to be increasing (2-3) and may now account for 20% to 45% of all CDI cases (4-5). The recent increase in mortality, morbidity and relapse rates of CDI has largely been associated with one strain of _C. difficile_, ribotype 027/North American pulsotype 1 (NAP1)/toxinotype III (4-9). However, ribotype 027/ _C. difficile_ has largely been associated with one strain of _C. difficile_ associated diarrhea and pseudomembranous colitis (1). Although _C. difficile_ infection (CDI) occurs mostly in health care facilities and is considered to be a nosocomial infection, the rate of community-acquired _C. difficile_ infection (CA-CDI) appears to be increasing (2-3) and may now account for 20% to 45% of all CDI cases (4-5). The recent increase in mortality, morbidity and relapse rates of CDI has largely been associated with one strain of _C. difficile_, ribotype 027/North American pulsotype 1 (NAP1)/toxinotype III (4-9). However, _C. difficile_ isolated from CA-CDI belong to a range of different _C. difficile_ types.

Several studies that identified _C. difficile_ contamination in retail meat have reported a predominance of ribotype 027/NAP1/toxinotype III and ribotype 078/NAP1-B/toxinotype V strains (1,4,6-7,10-11). Although previous Canadian studies have described the presence of _C. difficile_ spores in meat products purchased in Ontario, Quebec and Saskatchewan (6,10), no Manitoba data have yet been available. Here, we report the occurrence and characteristics of _C. difficile_ in retail meat sold in Winnipeg, Manitoba.

METHODS

Ground beef and ground pork were purchased from three major food chains and three local meat shops in Winnipeg over a four-week period in January 2007. Every week, one beef and one pork sample were collected from each store. In total, 48 meat samples were tested for the presence of _C. difficile_ spores. The meat was purchased in the smallest available volume and was stored at refrigeration temperature (2°C to 8°C) until processed. For each meat sample, 20 g were placed into a sterile 50 mL tube and processed using an alcohol meat treatment protocol to eliminate vegetative bacteria. Briefly, 5 mL of sterile reverse osmosis (RO) water and 20 mL of 95% ethanol were added to the meat. The blend was mixed and kept at room temperature for 40 min, followed by centrifugation at 3000 rpm for 15 min at 4°C. The supernatant was discarded and the pellet was resuspended in 30 mL of sterile RO water. The mixture was then filtered using a coffee filter placed inside a sterile funnel to remove gross particles. The filtrate was centrifuged at 3000 rpm for 15 min at 4°C and the pellet was suspended in 100 μL of sterile RO water. This entire sample was then inoculated onto _Clostridium difficile_ Moxalactam Norfloxacain (CDMN) agar medium. The plates were examined for growth after 72 h of anaerobic incubation at 35°C to 37°C. Suspected colonies (grey to pinkish-white in colouration with a distinct ‘horse manure’ odour) were enumerated and the presence of _C. difficile_ was confirmed with Gram staining, latex agglutination and a fluorescent appearance under ultraviolet light. The identity of _C. difficile_ isolates were also confirmed by the presence of the triose phosphate isomerase (tpi) housekeeping gene (12). The isolates were further tested for the presence of tcdA (enterotoxin gene), tcdB (cytotoxin gene), tcdC (negative regulatory gene). Pulsed-field gel electrophoresis (PFGE)-SmaI was performed at the National Microbiology Laboratory (NML) (Winnipeg, Manitoba) to determine the pulsotype of _C. difficile_ isolates. The susceptibility of these three _C. difficile_ isolates to metronidazole, vancomycin, clindamycin, rifampicin, moxifloxacain and tigecycline was also tested at NML by E-test. Resistance to antimicrobials was determined based on the Clinical and Laboratory Standards Institute (Wayne, USA) (13-14) and United States Food and Drug Administration guidelines (15).

To determine how many spores needed to be present in 20 g of ground meat such that at least a single _C. difficile_ colony could be detected on CDMN plates, the limit of detection for the alcohol meat treatment protocol was determined. A known number of spores (2×10⁷ obtained from _C. difficile_ strain 765 clinical isolate) were added to 20 g of a meat sample that was culture negative for _C. difficile_. The meat sample was then processed using the alcohol treatment protocol. The final alcohol-treated sample was used to prepare serial 1:10 dilutions. One hundred microliters of each dilution was inoculated and spread over the surface of a CDMN plate. After 72 h of anaerobic incubation at 35°C to 37°C, _C. difficile_ colonies were counted and the limit of detection was calculated.

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C difficile in retail meat

<table>
<thead>
<tr>
<th>C difficile isolate</th>
<th>Metronidazole</th>
<th>Vancomycin</th>
<th>Clindamycin</th>
<th>Rifampycin</th>
<th>Moxifloxacin</th>
<th>Tigecycline</th>
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<tbody>
<tr>
<td>EH-4</td>
<td>0.125</td>
<td>0.5</td>
<td>0.75</td>
<td>&lt;0.002</td>
<td>1</td>
<td>0.064</td>
</tr>
<tr>
<td>FH-4</td>
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<td>0.5</td>
<td>0.75</td>
<td>&lt;0.002</td>
<td>1</td>
<td>0.064</td>
</tr>
<tr>
<td>CP-1</td>
<td>0.125</td>
<td>0.5</td>
<td>&gt;256</td>
<td>&lt;0.002</td>
<td>&gt;32</td>
<td>0.094</td>
</tr>
</tbody>
</table>

Table 1: Antimicrobial susceptibility of three Clostridium difficile strains isolated from retail meat sold in Manitoba

*p<Only the >256 μg/mL for clindamycin (resistant at ≥8 μg/mL) and >32 μg/mL for moxifloxacin (resistant at ≥8 μg/mL) are considered resistant; all other minimum inhibitory concentrations are considered sensitive*

RESULTS

C difficile was isolated from 6.3% (3 of 48) of retail meat samples. C difficile isolates were detected in two ground beef samples (two of 24, [8.3%]) and one ground pork sample (one of 24, [4.2%]). Each C difficile isolate was from meat purchased at different stores, but the two beef isolates were from meat that was purchased during the same week. The limit of detection for the alcohol treatment protocol was 4 spores/g of ground meat.

All three C difficile isolates were positive for toxin A (tcdA) and toxin B (tcdB). Only one of the beef isolates (EH-4) was positive for binary toxin and had an 18 bp deletion in tcdC. This isolate belonged to NML PFGE type 0088 (Figure 1). The other beef isolate (FH-4) was NML PFGE type 0348. Both beef-isolated C difficile organisms were sensitive to all antimicrobial agents tested (Table 1). The only C difficile strain isolated from ground pork (CP-1) belonged to NML PFGE type 0139. This pork-isolated C difficile was resistant to both clindamycin (resistant at ≥8 μg/mL) and moxifloxacin (resistant at ≥8 μg/mL) (13-14) (Table 1).

In total, approximately 4800 C difficile strains have been typed at NML of these, 588 were from Manitoba. Of the 588 Manitoba isolates, 0.51%, 0.34% and 3.74% were pulsotypes 0288, 0348 and 0139, respectively (Figure 2). The 588 Manitoba isolates represent 140 unique PFGE types, 62 of which were exclusive to this province. Overall, 4.6% of total Manitoba pulotype isolates had pulotypes similar to C difficile isolated from retail meat in the present study. Pulsotype 0288 has been previously detected in two isolates from Manitoba, as well as five isolates from other provinces (Ontario and Alberta), whereas pulotypes 0348 and 0139 have only been isolated in Manitoba.

In the present study, we found C difficile in 6.3% of ground meat products in Winnipeg. Despite the fact that we analyzed only 48 meat specimens, our results were similar to those reported by other groups. Rodriguez-Palacios et al (10) conducted an across-Canada meat study and reported a C difficile prevalence of 6.1%. Molecular typing of our C difficile isolates from retail meat in Manitoba revealed that all of these strains have pulotypes identical to strains previously isolated from patients with CDI in Manitoba. Finding strains of C difficile in meat with identical pulotypes to those isolated from humans (9) suggests that meat could be a source of transient but frequent ingestion of C difficile and raises a concern regarding food as a source for C difficile acquisition that may lead to CDI (ie, CDI as a zoonosis).

DISCUSSION

In general, C difficile can be found relatively commonly in various meat products, and strains isolated from retail meat share similar PFGE fingerprints with those isolated from CDI patients (1,11).
Different sampling and isolation methods could account for differences in *C. difficile* prevalence rates in meat, ranging from 2.4% (8) to 47.8% (4). However, these studies demonstrate that the presence of low levels of *C. difficile* in retail meat is a common occurrence throughout many different geographical regions (7,10). Differences in meat handling in various regions may also contribute to variations in spore detection rates. Our data support the assessment reported by Weese (1) that because the prevalence of CA-CDI is not as common as the prevalence of *C. difficile* in retail meat, the existence of an obligatory association between ingestion of *C. difficile* and CA-CDI is unlikely. In other words, ingestion of *C. difficile* does not automatically lead to CDI and, in the majority of cases, it is likely that the presence of *C. difficile* spores in the gastrointestinal tract of humans is transient. However, it is difficult to overlook the fact that strains of *C. difficile* with the same pulsotypes are found in meat and patients with CDI. It is likely that additional factors (e.g., concurrent antibiotics and lack of immune protection) are necessary before the *C. difficile* spores present in meat can cause infection. The possibility of foodborne transmission of *C. difficile* and conditions under which such *C. difficile* ingested from meat develops into CDI warrants further investigation.

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REFERENCES