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Short communication

DETECTION OF TOXIGENIC *CLOSTRIDIUM DIFFICILE* STRAINS ISOLATED FROM MEAT AND MEAT PRODUCTS IN IRAN

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Summary

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The aim of this study was to determine the occurrence of toxigenic *C. difficile* in some of the meat products in Iran. Samples of hamburger (100), minced beef meat (150), chicken nugget (150), sausage (100) and canned meat (70) were collected from the retail trade and analysed for the presence of *C. difficile* using selective enrichment in *C. difficile* broth, subsequent alcohol shock-treatment and plating onto *C. difficile* selective medium. *C. difficile* isolates were tested for determination of toxins A and B production by enzyme linked immunosorbent assay. Of 570 samples tested, six (1.2%) were positive for the presence of *C. difficile*: one from hamburger (1.0%) and five from minced beef meat (3.3%). Among six *C. difficile* isolates five (83.3%) were found to be toxigenic and were positive for toxin A and/or B. This study shows the importance of minced beef meat as potential source of *C. difficile* infection in people who consume it.

Key words: *Clostridium difficile*, meat, meat products, toxigenic strains

Clostridium difficile is recognised as a nosocomial pathogen associated with antimicrobial drug-associated diarrhoea and pseudomembranous colitis in humans and the infection is believed to be acquired nosocomially. *C. difficile* has also been shown to be an important pathogen causing diarrhoea in humans in communities outside hospital environments (Chernak *et al.,* 2005). Reports from Canada, USA and Europe indicate that a large proportion of these cases of community-acquired *C. difficile* infection (CA-CDI) are not linked to recent antibiotic therapy, older age, significant comorbidity or previous hospitalisation (Wilcox *et al.,* 2008; Bauer *et al.,* 2009). In addition, recent epidemiological data indicate that the incidence and severity of the disease appear to be increasing worldwide (MacCannel *et al.,* 2006).

Toxigenic strains of *C. difficile* typically produce 2 major toxins, A and B, although a small percentage produce only toxin B (Pituch *et al.,* 2006). Certain strains may also produce a binary toxin

(known as CDT), whose clinical relevance is under investigation. Ribotype 027 and 078 strains produce all 3 toxins. This strain has been implicated in recent outbreaks of severe disease internationally and is also a common endemic strain in many regions (Goorhuis *et al.,* 2007; Hubert *et al.,* 2007; Songer *et al.,* 2009).

Food animals are an important source of human enteropathogenic microorganisms and can be spread to men through consumption of foods of animal origin. In accordance herewith, recent reports show a remarkable overlap between isolates from animals and humans (Goorhuis *et al.,* 2008). In recent studies *C. difficile* has been isolated from several food animal species and variety of foodstuff, sharing high level of genetic similarity with those of human isolates (Simango, 2008; Rupnik *et al.,* 2009; Weese, 2010).

The epidemiology of *C. difficile* infection (CDI) in Iran is essentially unknown. Although toxigenic *C. difficile* isolation has been reported from Iranian hospitals (Jalali *et al.,* 2012), to the authors' knowledge, there is limited information regarding the prevalence rate of *C. difficile* in foodstuff in Iran (Esfandiar *et al.,* 2014; Rahimi *et al.,* 2014a,b). The present study was conducted to determine the prevalence of toxin producing *C. difficile* strains isolated from retail meat products in Isfahan, Iran.

From April to October 2012, a total of 570 meat product samples including hamburger (n=100), minced beef meat (n=150), chicken nugget (n=150), sausage (n=100) and canned meat (n=70) were purchased from randomly selected retail stores in Isfahan and Shahrekord cities in the center of Iran. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler with ice packs and processed within 6 h.

The detection and isolation method used was based on the method described by Rodriguez-Palacios *et al.* (2007) and de Boer *et al.* (2011). Briefly, 5 g of each sample was transferred to 20 mL of *C. difficile* broth (CDB), containing *C. difficile* selective supplement (Oxoid SR0173) and 5% (v/v) defibrinated sheep blood. After inoculation at 37 °C for 10 to 15 days under anaerobic conditions, 2 mL of the enriched broth was added to 2 mL of 96% ethanol in a centrifuge tube and homogenised during 50 min on a shaker. After centrifugation (3800×g for 10 min), a loopful material from the sediment was streaked onto *C. difficile* agar base (Oxoid CM0601) supplemented with an antibiotic supplement for the selective isolation of *C. difficile* (Oxoid SR0174) and 7% (v/v) defibrinated sheep blood, and the plates were incubated for 24 to 48 h at 37 °C, under anaerobic conditions. Up to three colonies per plate were subcultured onto tryptone soya agar (Oxoid CM0131) and tested by standard microbiological and biochemical procedure (Harvey *et al.,* 2011). Crudely extracted DNA (boiling, 10 min) was used for PCR confirmation (*tpi* gene detection) of isolates as performed in previous studies (Rodriguez-Palacios *et al.,* 2009).

The isolates confirmed as *C. difficile* were cultured on sheep blood agar under anaerobic conditions at 37 °C for two days. After culture, a thick bacterial cell suspension was prepared in 1 mL universal stool buffer (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) and centrifuged at 3000×*g* for 10 min. The supernatants were tested for the presence of *C. difficile* toxins A or B by enzyme linked immunosorbent assay (ELISA) detection kit (RIDASCREEN, R-Bio-

pharm AG, Darmstadt, Germany) as per manufacturer's instructions. Positive and negative controls were included in each batch.

The results of the prevalence testing are summarised in Table 1. In total, six of 570 (1.2 %) samples were *C. difficile* positive. *C. difficile* strains were isolated from a sample of hamburger (1.0%) and from five samples of minced beef meat (3.3%), but not from any of the samples of chicken nugget, sausage and canned meat. Among the six *C. difficile* isolates five (83.3%) were found to be toxigenic for toxin A and/or B. Recently published studies report the isolation of *C. difficile* from meat and meat products in relatively high percentages, ranging from 2.4 to over 42% of samples collected at retail (Simango & Mwakurudza, 2008; Rodriguez-Palacios *et al.,* 2009; Von Abercron *et al.,* 2009; Weese *et al.,* 2010). In a recent study in Iran, toxigenic *C. difficile* were detected from 2/7 (28.5%) hamburger processing plants, in 3/54 (5.6%) of beef meat samples, 2/56 (3.5%) of swabs taken from the environment and 4/56 (7.1%) of hamburger samples after both molding and freezing (Esfandiar *et al.,* 2014). In a study reported from Iran by Rahimi et al. (2014a), 13 of 660 meat samples (2%) including buffalo, beef, cow, sheep, and goat meat were contaminated with *C. difficile*. The highest prevalence of *C. difficile* was found in buffalo meat (9%). In another study conducted in Isfahan, Chaharmahal va Bakhtiari, and Khuzestan province of Iran, *C. difficile* was identified in 1.43% of 135 bulk milk samples (Rahimi *et al.,* 2014b).

The first specific investigation of *C. difficile* contamination of retail meat intended for human consumption was a study from Canada in 2007 (Rodriguez-Palacios *et al.,* 2007). By enrichment culture, *C. difficile* was isolated from 12 of 60 (20%) samples (21% ground beef and 14% ground veal). The most common strain, accounting for 67% of isolates, was a toxigenic strain that possessed genes encoding TcdA, TcdB and CDT, belonging to toxinotype III. A similar study from the USA, using convenience sampling from stores in the Tuscon, Arizona area, reported isolation of *C. difficile* from 37 of 88 (42%) samples, including ground beef (13/26, 50%), summer sausage (1/7, 14%), ground pork (3/7, 43%), braunschweiger (10/16, 63%), chorizo (3/10, 30%), pork sausage (3/13, 23%) and ground turkey (4/9, 44%) (Songer *et al.,* 2009). In another study in Pittsburgh, Pennsylvania, USA, the isolation rates of *C. difficile* in ground meat and sausage samples were 2% (2 of 102) (Curry *et al.,* 2012). In contrast, *C. difficile* was not

Table 1. Prevalence of *Clostridium difficile* detected in meat product samples in Iran

Meat products	Number of samples	Number of *C. difficile*-positive samples	Number of isolates positive for toxins A and/or B
Hamburger	100	1 (1.0%)*	1 (1.0%)
Minced meat	150	5 (3.3%)	4 (2.7%)
Chicken nugget	150	0	0
Sausage	100	0	0
Canned meat	70	0	0
Total	570	6 (1.2%)	5 (0.9%)

*Results expressed as number of *C. difficile*-positive samples/number of samples analysed (%).

isolated from any of 51 beef, 27 pork and six chicken samples in Austria (Indra *et al.,* 2009). Also, Jöbstl *et al.* (2010) reported a low prevalence of 3% for *C. difficile* in retail ground meat samples; positive samples could only be found among mixed beef and pork samples, but not among ground beef (Jöbstl *et al.,* 2010).

The source of *C. difficile* in meat is not clear. Carcasses may become contaminated with faecal material or from the environment during the slaughtering process. Contamination at retail level may also occur from the environment or through transmission by food handlers. The excellent survival of *C. difficile* spores in the environment increases the possibilities for contamination of animals and foods.

Quantification of *C. difficile* contamination in retail meat showed that generally low numbers, ranging from 60 to 240 spores/g, can be found (Weese *et al.,* 2010). The meaning of this is not clear, as the infectious dose for *C. difficile* is not known. This and other published studies show that there is a potential for transmission of *C. difficile* to humans via the food chain. Also, this study shows the importance of minced beef meat as potential source of *C. difficile* infection in people.

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