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ORIGINAL ARTICLE

# Prevalence of *Clostridium difficile* in raw cow, sheep, and goat meat in Jazan, Saudi Arabia



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## KEYWORDS

*Clostridium difficile*;  
Raw meat;  
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**Abstract** *Background:* *Clostridium difficile* has been shown to be a nosocomial infection associated with diarrhoea and pseudomembranous colitis in hospitalized patients especially old patients. In my previous studies, it was shown the occurrence of *C. difficile* in animals feces and vegetables which may act as a source of infection to humans.

The aim of the study was to determine the prevalence of *C. difficile* in retail raw cow, sheep, and goat, meat in Jazan, Saudi Arabia.

*Method:* A total of 600 raw meat samples from cow, sheep, and goat were collected during June–December 2015, and tested for the presence of *C. difficile*. The method used to check for the presence of *C. difficile* was by choosing selective enrichment media in *C. difficile* broth, followed by alcohol shock-treatment and plating onto *C. difficile* selective medium. *C. difficile* isolates were typed using PCR ribotyping and also analyzed for antibiotic susceptibility.

*Results:* It was shown that, 9 of 600 meat samples (1.5%) were contaminated with *C. difficile*. The prevalence of *C. difficile* was as follow: 7 out of 600 (1.17%) were found in cow, 2 out of 600 (0.3%) were found in sheep, while was no *C. difficile* was isolated from goat. Eleven out of 18 *C. difficile* isolates were positive for *tcdA*, *tcdB* and *cdtB* toxin genes and were classified as ribotype 078. Three strains were positive *tcdA*, and *tcdB*, and two strains possessed only *tcdB*. *C. difficile* strains showed high resistance to ampicillin, gentamycin, erythromycin and nalidixic acid.

*Conclusions:* The present work shows the potential risk of raw meet in transmitting *C. difficile* to humans.

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## 1. Background

*Clostridium difficile* is a Gram-positive, anaerobic bacterium. For the last few years, *C. difficile* infection has become a major cause of nosocomial infection. Although it is known as health-care infection, the incidence of community-acquired infections has increased recently, such as infirmity (McFarland et al., 2007). Notably, there is a clue that *C. difficile* may be transmitted to the healthcare by asymptomatic carriers (Clabots et al.,

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1992). There are reports about the asymptomatic carriers of *C. difficile* in healthy people which vary from low percent 0–3% in Europe to a high percent which is 15% in Japan (Mulligan, 2008). Up to date a small amount of knowledge is known about the prevalence of *C. difficile* in the community and the possible way of transmission to humans.

*C. difficile* was found in many sources, including food, soil, water, and animal feces (Rodriguez-Palacios, 2007; Al Saif and Brazier, 1996) and this could suggest the possible way of transmission to human. *Salmonella* species and *Escherichia coli* O157 were implicated in food infections (Sagoo et al., 2003; Delaquis et al., 2007).

We have shown earlier the prevalence of *C. difficile* in ready to eat salads distributed in the markets in UK (Bakri et al., 2009) and it was the first study world-wide isolating *C. difficile* from ready to eat salads. In this study we are trying expand the search to find another source of *C. difficile* transmission to human.

The prevalence of *C. difficile* transmission from animals to human is not known in Saudi Arabia especially in Jazan province. The aim of this study was to determine the prevalence of *C. difficile* in raw cow, sheep, goat, and camel meat in Jazan, Saudi Arabia.

## 2. Methods

### 2.1. Sample collection

A total of 600 raw meat samples were collected between June and December 2015. The number of samples collected from each animal was 200 samples from cow, 200 samples from sheep, and 200 samples from goat. The samples were purchased from different shops in meat market in Jazan, Saudi Arabia. Each sample was purchased, placed in sterile bag, and immediately taken to the laboratory for processing.

### 2.2. Isolation and identification of *C. difficile*

On arrival, the samples were processed immediately using aseptic techniques. The method for detection used was based on the method described by Rodriguez-Palacios et al. (Rodriguez-Palacios (2007)). In brief, 5 g of each sample was transferred to 20 mL of *C. difficile* broth (CDB; Oxoid SR0048) containing 40 g/l proteose peptone, 5.0 g/l, disodium hydrogen phosphate, 0.1 g/l magnesium sulfate, 2.0 g/l sodium chloride, 6.0 g/l fructose and 1.0 g/l sodium taurocholate supplemented with *C. difficile* selective supplement (Oxoid, UK, Code: SR0173) and 5% (v/v) defibrinated sheep blood. After incubation at 37 °C for 10–15 days under anaerobic conditions 2 mL of the enrichment broth was added to 2 mL of 96% ethanol in a centrifuge tube and homogenized for 50 min on a shaker at room temperature. After centrifugation (3800g for 10 min), a loopful of the sediment was streaked onto *C. difficile* agar base (Oxoid, UK, Code: CM0601) supplemented with an antibiotic supplement for the selective isolation of *C. difficile* (Oxoid, UK, Code: SR0173) and 7% (v/v) defibrinated sheep blood and the plates were incubated for 48 h at 37 °C, under anaerobic conditions. Three colonies per plate were subcultured onto tryptone soya agar (Oxoid, UK, Code: CM0131) and tested by standard microbiological and biochemical procedures including odor, Gram stain morphology and L-proline.

Crudely extracted DNA (boiling for 10 min) was used for PCR confirmation (housekeeping *tpi* gene detection), and determination of toxin gene (*tcdA*, *tcdB* and *cdtB*) of isolates as performed in previous studies.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility analysis was conducted by using Kirby–Bauer disk diffusion method using Mueller–Hinton agar as described by Rodriguez-Palacios (2007). In the present study we have tested the following antimicrobial agents: nalidixic acid (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), tetracycline (30 µg), doxycycline (30 µg), gentamicin (10 µg), metronidazol (5 µg), ampicillin (10 µg), chloramphenicol (30 µg), vancomycin (30 µg), Piperacillin-tazobactam (10 µg), Cefoxitin (15 µg) and clindamycin (2 µg). Inoculated plates were incubated at 37 °C for 48 h, under anaerobic conditions. The susceptibility of the *C. difficile* to each antimicrobial agent was measured and the results were interpreted as described by Clinical and Laboratory Standards Institute, 2007.

## 3. Results and discussion

The results show the prevalence of *C. difficile* isolated from cow, sheep, and goat meat in Jazan, Saudi Arabia (Table 1). 9 *C. difficile* isolates out of 600 meat samples were identified. There was a significant difference ( $P < 0.05$ ) in the frequency of positive samples among the meat samples.

The highest prevalence of *C. difficile* was found in cow meat samples (7 out of 200), followed by sheep (2 out of 200), and there were no *C. difficile* isolates were identified from goat (Table 1).

The difference in the number of *C. difficile* isolates between cows and sheep could be due to the fact that in our culture the farmers keep cows most of the time in a cowshed which is usually near to their houses. Moreover, farmers get in touch with cows on a daily basis to get the milk and this increase the chance of *C. difficile* transmission between human and cows. On the other hand, sheep usually leave the barn early morning and return in the evening which indicates there is less time compared to cows to be in touch with humans. Finally, goats usually live in mountains areas where they move freely with less contact with humans. This could explain the absence of *C. difficile* isolates from goat samples.

The source of *C. difficile* in meat samples is unclear. The source of *C. difficile* in meat samples could be the gastrointestinal tract of animals or due to cross contamination from the slaughters handling the meat (Keessen et al., 2011).

Eleven out of 18 *C. difficile* isolates were positive for *tcdA*, *tcdB* and *cdtB* toxin genes and were classified as ribotype 078. Three strains were positive *tcdA*, and *tcdB*, and two strains possessed only *tcdB*. The remaining two isolates were proved

**Table 1** Prevalence of *Clostridium difficile* detected in cow, sheep, and goat meat samples.

Type of sample	Number of samples	Number of <i>C. difficile</i> positive samples
Cow	200	7 (3.5%)
Sheep	200	2 (1%)
Goat	200	0
Total	600	9 (1.5%)

**Table 2** Antimicrobial resistance of 18 *Clostridium difficile* isolated from beef, cow, sheep and goat, meat in Jazan, Saudi Arabia.

Antimicrobial agent	Sensitive	Intermediate	Resistant
Ampicillin	2 (11.11)	5 (16.67)	13 (72.22)
Chloramphenicol	16 (88.89)	2 (11.11)	0 (0)
Ciprofloxacin	7 (38.89)	6 (33.33)	5 (27.78)
Doxycycline	8 (44.44)	10 (55.56)	0 (0)
Clindamycin	4 (22.22)	8 (44.44)	6 (33.33)
Erythromycin	3 (16.67)	6 (33.33)	9 (50.00)
Gentamicin	0 (0)	3 (16.67)	15 (83.33)
Metronidazole	17 (94.44)	1 (5.56)	0 (0)
Nalidixic acid	1 (5.56)	3 (16.67)	14 (77.78)
Tetracycline	8 (44.44)	5 (27.78)	5 (27.78)
Vancomycin	12 (83.33)	3 (16.67)	0 (0)
Piperacillin-tazobactam	4 (22.22)	8 (44.44)	6 (33.33)
Cefoxitin	9 (50.00)	5 (27.78)	4 (22.22)

to be nontoxigenic. The majority of ribotype 078 is consistent with other studies of food animals and food (Indra et al., 2008). Presence *C. difficile* ribotype 078 strain in humans in the same region with CDI, attention must be given as the main source might be food. Yet, further research is needed to conclude whether food is rational source of this particular bacterial prevalence.

Antimicrobial susceptibility analysis results are shown in Table 2. *C. difficile* strains showed high resistance to ampicillin, gentamicin, erythromycin and nalidixic acid. Most of the *C. difficile* isolates were susceptible to metronidazole, chloramphenicol and vancomycin as was observed in other studies (Jöbstl et al., 2010). Metronidazole and vancomycin are the most regularly used drugs to treat *C. difficile* diarrhea patients but they are not used in animal's food.

#### 4. Conclusion

This is the first work done on *C. difficile* prevalence in animal meat in Jazan, Saudi Arabia. Our work shows the potential risk of meat, as a source of transmitting *C. difficile* to humans. The presence of blood and absence of cleaning in Slaughterhouses can be an excellent source for spreading infections.

There is a high demand for good observation for slaughter hygiene, get rid of animal remnants in a healthy way, and implementation of good slaughtering practice.

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