Isolation and Characterization of *Clostridium difficile* in Farm Animals from Slaughterhouse to Retail Stage in Isfahan, Iran

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**Abstract**

To determine the prevalence of *Clostridium difficile* in farm animals from slaughterhouse through to retail stage, a total of 750 samples of feces, postvisceral and washed carcass were collected from cattle, camels, goats, and sheep in Isfahan, Iran. The overall prevalence of *C. difficile* in feces, postvisceral and washed carcass were 20 (13.3%), 23 (15.3%), and 11 (7.3%), respectively; while *C. difficile* was isolated from 79 (26.3%) retail samples. Twenty-nine (3.8%) isolates were toxigenic, with most toxigenic isolates (n = 17, 5.6%) identified from the retail stage. All toxigenic isolates harbored *tedA* and *tedB*, however, all were negative for *cdeB*. The 29 isolates were classified into 21 different ribotypes. This study revealed evidence of existence of toxigenic *C. difficile* in farm animal feces and meat in Iran.

**Introduction**

*CLOSTRIDIUM DIFFICILE INFECTION* (CDI) has been identified as an important cause of hospital-associated diarrhea in healthcare settings and community. The epidemiology of community CDI is poorly understood, with a lack of clear information on routes of bacterium transmission. This has led to consideration of different potential sources in the community in specific food. The hypothesis that *C. difficile* is a foodborne pathogen has been highlighted due to the genotypic overlap of *C. difficile* from humans with CDI and those isolated from food animals and retail meat. Recently, *C. difficile* has been reported in retail meat and meat-processing plants in Iran, with slaughterhouses identified as a possible source of this organism (Esfandiari et al., 2014a, b; Rahimi et al., 2014). Therefore, the aim of this study was to evaluate the prevalence of *C. difficile* in cattle, camels, goats, and sheep in slaughterhouse and retail meat in Isfahan, Iran.

**Materials and Methods**

A large slaughterhouse of cattle, camels, goats, and sheep in Isfahan province, Iran was enrolled. A total of 750 samples were collected during 12 slaughterhouse and retail visits between December 2012 and July 2013. At each visit, two to five animals per species were sampled. Feces were collected before slaughtering (n = 150). Animals were followed through the slaughtering line so that further samples could be collected from the same animals’ carcasses throughout processing. Samples of carcasses were taken at postvisceralization (n = 150) and postwashing (n = 150) steps. A further 300 samples of meat were collected from 11 butcher stores supplied from the slaughterhouse under study. Presumptive colonies of *C. difficile* recovered from samples were screened by L-proline test. For molecular characterization, isolates were screened for the presence of genes encoding *tpi*, *tedA*, *tedB*, and *cdeB*. *C. difficile* isolates were also subjected to polymerase chain reaction (PCR) ribotyping (Bidet et al., 2014a, b; Rahimi et al., 2014).